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EXPERIMENTAL APPROACHES TO THE STUDY OF DRUG DEPENDENCE

Edited by
HAROLD KALANT
ROSEMARY D. HAWKINS

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Proceedings of
an interdisciplinary research conference
held at the University of Toronto
March 1965
under the sponsorship of the
Alcoholism and Drug Addiction
Research Foundation of Ontario

Edited by

HAROLD KALANT M.D., PH.D.

ROSEMARY D. HAWKINS PH.D.

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Preface

During a visit to Toronto several years ago, Dr. Ebbe Curtis Hoff suggested that the North American Association of Alcoholism Programs, of whose research committee he was the chairman, should sponsor a research conference on current trends in alcoholism research. In the discussion stirred by his suggestion, it was agreed that there were at least two major aspects of this research which clearly merited a thorough review at expert level.

The first of these was the relation of different research approaches to each other. Alcoholism and other drug addictions offer an excellent opportunity for what has become fashionably known as 'interdisciplinary research'. An understanding of the addictive process involves an explanation of the interaction between subject and drug at behavioural, physiological and biochemical levels. Yet it was apparent that scientists working at these different levels all too often fail to understand each other, to see the mutual relevance of their work or to appreciate the advantages and limitations of the techniques which they borrow from each other.

The second major aspect which warranted full discussion was the comparison of the various drugs of dependence potential with each other. Clinical and social experience made it perfectly clear that the addictive process has broad common features which are relatively independent of the specific pharmacological properties of the individual drugs in question: alcohol, opiates, barbiturates, other hypnosedatives, minor tranquillizers and stimulants. To what extent did the actions of individual drugs modify or influence the process or characteristics of addiction?

It was agreed that a most profitable way to examine these questions would be to convene a small conference of experts drawn from the various fields. The intention was not to hold a symposium for the presentation of formal research papers, but rather, to provide an opportunity for informal discussion in which participants could freely question, challenge, inform and stimulate each other with new insights into their respective areas of research. To achieve this goal it would be necessary to limit the meeting to a relatively small size, so that the participants would be able to talk freely.

For various reasons it was not possible for the NAAAP to undertake the meeting. However, the potential benefits for researchers in the field of addictions appeared great enough that the Alcoholism and Drug Addiction Research Foundation of Ontario decided to sponsor the conference and publish its proceedings. The meeting was held at Massey College, in the University of Toronto, on March 18 and 19, 1965. Most of the time was devoted to a wide-ranging and unstructured conversation, arising from three working papers which dealt, respectively, with behavioural, neurophysiological and biochemical aspects of the topic. The entire conference was recorded on tape and transcribed later. The delay in publication is due in part to the mechanical problems of transcription, in part to the fact that the participants were subsequently given a lengthy opportunity to review and correct the original record and in part to the complexity of editing the verbatim record of an unstructured discussion and rearranging it into an organized series of topics. The editors accept, with due regret, the responsibility for any loss of spontaneity, continuity and vigor of the original discussion which may have resulted from the drastic rearrangement.

It will be clear from the foregoing remarks that the reader should not expect to find new research data in this publication, nor even an exhaustive review of the relevant literature. The emphasis is on a critical examination of the manner of conducting research on topics related to drug dependence and of the validity of conclusions which can be drawn from this research. The content is therefore as timely now as when the conference actually took place. Where certain specific views or suggestions may have been modified by later developments, or where work mentioned by the participants was subsequently published, the editors have inserted relevant references either in the text or as footnotes. However, these changes are minor, and in most cases do not affect the arguments advanced at the meeting.

It is a pleasure to acknowledge the invaluable role of Mr. V. H. K. Lang in planning and making all the practical arrangements for the conference; the excellent hospitality of the Master and Bursar of Massey College; the secretarial services of Mrs. E. Brown, Mrs. S. Jaychuk and Miss J. Dark in the original transcription and numerous revisions of the manuscript; the careful bibliographic check by Mrs. B. J. Constable; and, above all, the contribution of time and expert knowledge by the participants.

H. K.
R. D. H.

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Note on definitions

In the text that follows the terms 'ethanol' and 'alcohol' are used interchangeably. The term 'alcohols' is used to designate the homologous series of aliphatic alcohols.

The World Health Organization has recommended that the term 'Drug Dependence,' with specification of the type of drug involved, be used in place of the older term 'Drug Addiction.' At the time this conference took place, the older term was still well entrenched in popular and professional usage. As so used, it generally denoted a state characterized by physical dependence on the drug as well as an overpowering need to obtain and use it. Even though there are no sharp lines of division between such a state and less severe degrees of dependence, the term 'Addiction' does perhaps retain some validity as a designation for the most fully developed expression of drug dependence. It is used in this sense in the discussions which follow.

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EXPERIMENTAL APPROACHES TO THE STUDY OF DRUG DEPENDENCE

Behavioural effects of psychoactive drugs

ROGER W. RUSSELL
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INTRODUCTION

Essentially this paper is directed towards two kinds of issue: basic methodological problems, and problems involved in organizing our thinking about ways in which three classes of variables interact–drugs, biochemical events and behaviour. No attempt has been made to prepare a chronology or a summary of all the available research upon the effects of drugs on behaviour. Before I did a review of this latter kind for the *Annual Review of Psychology* (Russell, 1964a), I consulted with the National Medical Library and was told that if I wanted to cover all the references, I would be faced with between 10,000 and 15,000 papers published during the four years involved in the review. In my opinion, a great many of these are not worth consulting in any event, mainly because of methodological difficulties in the research involved. Thus, despite a very extensive literature it is still possible to draw but few general conclusions about the effects of drugs upon behaviour. This is due in part to the complexities of the variables involved, but it is also due to the level of methodological sophistication which has characterized past research in the field and which Professor Drew (1963), speaking about research on behavioural effects of ethanol, has described as being '. . . considerably lower than would be tolerated in almost any other field.' Within the last decade, the greatly expanded use of psychoactive drugs in the treatment of mental illness has had the effect of highlighting the methodological problems. A review of the literature suggests that modern research in behavioural neuropharmacology is emerging from a phase of development in which emphasis has rightly been placed upon the *means* for answering basic questions into a phase in which attention can be focused upon finding the *answers.* This being the case, it is probably more profitable to give greater emphasis in our present discussion to methodological issues than to the fragments of answers which can be culled from a large and, in many instances, contradictory literature.

From the behavioural point of view three general questions deserve the centre of the stage in research on any psychoactive drug:

1. What is the behavioural mode(s) of action of the drug? This question is analogous to the kinds of question asked by our colleagues in biochemistry and neurophysiology about the effects of drugs upon the variables with which they are concerned. There is, I am certain, a finite number of behaviour patterns, but I am not sufficiently bold as to try to list them. If one attempts to study the effect of drugs upon behaviour in a purely empirical way, it seems to me that there is just no end to the task. But it seems extremely important in any study of the behavioural effect of drugs to have some kind of general model on which to make decisions about what aspect of behaviour will be selected for study, and to concern oneself with something more than just the empirical kind of hypothesis that has in fact received, in the past, the most attention in the behavioural literature on drug research.
2. What quantitative relations exist between parameters of the drug and parameters of the behaviour affected? This follows naturally after the first question is answered. If one can establish that a particular mode of behaviour is affected by the drug, then this makes it possible to go to more quantitative studies of the relationships that exist.
3. What are the neurochemical and neurophysiological correlates of the behaviour affected?

In this working paper I propose to define each of these questions more precisely and to raise for discussion a number of issues involved in finding answers to them. A few introductory remarks are in order before getting on with my main task.

In his letter inviting me to prepare a working paper as a guide to discussion of ‘the behavioural aspect of the subject’, Dr. Kalant stated that ‘. . . one of the major purposes of the conference is to assess the significance of the chemical and physiological findings in relation to drug effects on behaviour.’ Psychologists have, in the past, tried to build up a systematic science of psychology in which they have been concerned with stimuli at the input end of their models and responses at the output end. Stimuli in psychopharmacology are chemical stimuli produced by the administration of the drug itself. But psychologists have always run into difficulty when they have limited their research purely to stimulus-response relations. No matter how careful a psychologist is in controlling the stimulating conditions, there often turns out to be considerable variability in the response, whichever one he may have selected for study. Clearly, events going on within the organism are extremely important in affecting the stimulus-response relations.

We must concern ourselves with three general properties of living organisms, i.e. neurochemical, neurophysiological, and behavioural. There are also two classes of hypotheses to consider. In the first class, drugs serve as independent variables and one of the three properties serves as dependent variables; hypotheses in this class are stated in the form: drug A affects neurochemical event X, or drug B affects neurophysiological event Y, or drug C affects behavioural event Z. The second class includes hypotheses in which drugs may serve as independent variables or as 'tools' in studying *interactions* between two or more of the properties. In this latter kind of hypothesis, the psychologist is viewing the organism as a living biological system which has several properties; an understanding of that system will depend upon his capability of relating all of these properties rather than just any selected two of them. This is a big order, particularly if one takes into consideration not only the possibility that drugs may serve as chemical stimuli affecting neurochemical events which in turn influence behaviour, but also that changes in behaviour may very well be accompanied by changes in neurochemical and neurophysiological events. For example, drug A stimulates neurochemical event X, changes in which are reflected in behaviour Z. Behavioural neuropharmacology continues to be concerned with testing hypotheses of both these classes. During the past ten years research oriented toward clinical applications has tended to focus more upon hypotheses of the first class, studying relations between the administration of a drug and its behavioural effects without concern about the events which intervene between the administration and the effects. More recently the belief that such relations will be more clearly understood and that, as a consequence, drugs will be more precisely selected or synthesized for the specificity of these behavioural effects, has led to an increasing interest in the search for neurochemical correlates of behaviour.

The point I wish to emphasize is that biochemical events and behaviour are reciprocally related in the sense that changes in one may be reflected in alterations of the other. Certainly when one considers some of the basic constructs in psychology that are necessary in any attempt to describe behaviour—the concept of memory for example—there has to be some lasting effect of behaviour upon some neurochemical or neurophysiological event or neuroanatomical structure in the organism to account for the carryover effects that occur as a result of experience and may last for a lifetime. Drugs do not introduce new processes in the organism; they affect the *neurochemical event* $\rightleftharpoons$ *behaviour* relations which already exist or may be formed under their influence.

Regardless of which class of hypotheses may be under test in any specific research project, there is, stated or implied, some model of how the major variables interact. A general model, which appears to fit our present knowledge, involves a reciprocal relation between neurochemical events and behaviour:

$$\begin{tikzcd} \text{Drugs} \arrow[r] & \begin{array}{c}\text{Neurochemical}\\ \text{Events}\end{array} \arrow[r, shift left, "1"] & \text{Behaviour} \arrow[l, shift left, "2"] \end{tikzcd}$$

The relation is reciprocal in the sense that: (1) drugs, as chemical stimuli, may produce changes in particular neurochemical events which in turn alter behaviour and (2) behaviour may produce persisting changes in neurochemical events, thereby affecting their responses to stimulation by drugs. This general model will help in organizing the discussion which follows.

BEHAVIOURAL MODES OF ACTION

Specificity of drug action is a central concept in all pharmacological inquiry and speculation and a basic objective in pharmacotherapy. The concept is clearly involved when questions are asked about the particular effect(s) a drug may have upon behaviour or about the differential effects which may be produced by different drugs. When one attempts to study the implications of the general model outlined in the introduction, one immediately runs into a number of methodological problems. What kinds of behaviour patterns should be selected for investigation, and upon what bases should they be chosen? The choice of behavioural measures selected for study, in any particular experiment involving effects of drugs, may be dependent upon some theoretical model or system in which the experimenter is interested. The choice may arise from some analysis he has already done or some form of criterion behaviour (such as the behaviour of the addict, the behaviour of the schizophrenic, the behaviour of some more highly evolved organism) which he is particularly interested in studying and which he hopes to be able to predict from studies of comparative nature. Some specific examples of the search for behavioural modes of action of drugs should help to focus attention upon the basic methodological problems with which the investigator is faced.

One approach to the search has been to compare the effects of several different drugs on behaviour in one carefully standardized test situation. This approach is illustrated in a series of studies using continuous avoidance as the behavioural baseline (Heise and Boff, 1962). This particular behaviour pattern was selected on the basis of a preliminary study which showed it to be very stable and to be sensitive to the effects of both stimulant and depressant drugs. Rats were required to press a lever which postponed the occurrence of shock for 40 seconds; trained subjects pressed the lever at a steady rate and received only occasional shocks. The test situation provided several measures of behaviour, one of which separated chlordiazepoxide and its analogs from hypnotics and primary muscle relaxants but did not distinguish clearly between the latter and hypnotics.

Another approach in the search for behavioural modes of action is illustrated in a series of studies in which several behavioural baselines as well as several different drugs were involved (Cook, 1964). Among the baselines were some maintained by positive reinforcement (food) and others by negative reinforcement (shock). The results clearly showed qualitative differences between the effects of the three drugs: chlorpromazine, meprobamate, and chlordiazepoxide. For example, behaviour maintained by negative reinforcement was inhibited by chlorpromazine, chlordiazepoxide increased the number of shocks received by the subjects but did not inhibit overall response rate, and meprobamate had only very weak effects.

In the research I have just described, the behavioural baselines selected were characterized primarily by one major feature of the test situation in which they were generated and maintained: the nature of the reinforcement. In a third approach the selection of behavioural measures is oriented toward quite a different criterion, i.e., toward a theoretical construct which may be part of some model of behaviour or may represent an abstraction from a variety of behaviour patterns. Test situations are selected which are purported to generate behaviour patterns from which generalizations relevant to the construct may be made. The behavioural modes of action of a drug are then stated in terms of the construct rather than in terms of the behaviour patterns observed directly. The use of theoretical constructs has characterized efforts to develop systematic bodies of knowledge in all areas of scientific thought. Psychology has a number of them, e.g., ‘memory’, ‘learning’, ‘emotion’, ‘motivation’, all of which are abstractions from empirical observations. ‘Fear’ is another such construct (Miller, 1964). It has received particular attention in a series of experiments (Miller and Barry, 1960) using a wide variety of test situations which included avoidance conditioning, potentiated startle response, approach-avoidance conflict and the conditioned emotional response. One example of the many results reported describes the effects of sodium amytal as significantly decreasing the disrupting effects of 'fear' in a conflict situation.

I have chosen these examples to illustrate different approaches to the search for behavioural modes of drug action. Although different in certain important regards, they all have certain methodological problems in common and it is to these that I wish to turn next.

Selecting Behaviour to be Measured

It takes but little thought to realize that the search for behavioural modes of action is complex, for the number of different behaviour patterns is large and it increases with increasing complexity of the organism. Obviously it would be folly to entertain for a moment the possibility of studying *all* the behaviour patterns of which even relatively simple organisms are capable. The basic issue here is analogous to that faced by our other partners in neuropharmacology. On what grounds can particular neurochemical events be selected as likely candidates for involvement in a drug's neurochemical mode of action? Where in the complexities of the nervous system should we look for neurophysiological effects? Several criteria have evolved for the selection of particular behaviour patterns to be measured.

1. *Analysis of criterion behaviour.* This approach begins with the analysis of human behaviour, e.g., 'addiction', or schizophrenia, for its basic characteristics. It proceeds to devise procedures for generating, in other species, behaviour patterns which have similar characteristics. The effects of experimental treatments, e.g., administration of drugs, on various parameters of the behaviour patterns selected have then been studied. Human behaviour has served as the criterion for selecting behaviour patterns to be studied in other species. The major difficulty in this approach lies in the analysis of the criterion behaviour; particularly when, as in the case of schizophrenia, the etiology of the behaviour is unknown.

2. *Analysis of criterion agents.* A second approach used frequently in preclinical psychopharmacology has involved analysis of the effects of criterion drugs upon behaviour. Certain effects on human behaviour of a number of chemical agents are already known. By studying effects of these agents on a variety of behaviour patterns in infrahuman

animals, identifiable profiles of effects have been established for different agents or classes of agents. Such profiles can be useful in research on homologues of the drugs upon which the original analysis of behaviour was based (McIlwain, 1964). It has also been reported (Janssen, 1964) that extrapolation from animal data to human effects on the basis of such profiles has been made with accuracy.

3. *Construct analysis.* A third approach has involved predictions from some theoretical model of behaviour. Such models consist of explanatory constructs from which predictions about behaviour can be made. Among the predictions are those which suggest that drugs with certain pharmacological modes of action should have specified effects upon behaviour. Techniques are selected for measuring the relevant behaviour patterns. When predictions prove to be accurate, the results of such research have been used as support for the validity of the theoretical model.

4. *A priori selection.* Another approach begins with a classification of behaviour based upon rational or empirical, e.g., factor analytic, grounds without involving a theoretical model. Behaviour patterns which are 'representative' of some or all classes have then been selected for study.

5. *Empirical interest.* Particular behaviour patterns have been selected for study solely on the grounds that the investigator has an interest in those particular forms of behaviour. No theoretical considerations are involved nor implications about the relations of one behaviour pattern to another.

Generating the Behaviour Pattern to be Measured

After selection of a behaviour pattern to be measured, problems arise in standardizing a procedure for generating that pattern under conditions in which it can be measured. How can the behaviour be elicited under conditions which are consistent from subject to subject and from trial to trial, and which minimize interference from variables that may later confound the interpretation of results? As in the selection of variables to be measured, the problems here are basically no different from those faced by investigators concerned with the measurement of neurochemical or neurophysiological effects of drugs. Measuring devices are applied by research workers; both the characteristics of an instrument and the skill of the worker in using it contribute to the reliability of the over-all measuring system and, hence, to the experimental error of any set of measurements. An instrument may not be sufficiently precise to measure what it is supposed to measure; it may have such a large experimental error that differences which in fact exist are not discernible.

The psychologist is sometimes thought of, particularly by those who have not worked with him closely, as being concerned either with variables that resist quantification or with variables that any intelligent layman is capable of measuring. As one of my friends once put it, psychology is often regarded as 'the study of the *id* by the odd'. The fact is that the psychologist has acquired a considerable sophistication in developing measuring instruments for behavioural variables, which are valid and reliable. The failure of some of his colleagues from other disciplines to recognize the existence of an extensive body of knowledge about the measurement of behaviour often dismays him. But it is there for consultation whenever behavioural variables are involved in research plans.

Behavioural Test Situations

Summaries of the research literature reveal the great variety of test situations which have been employed in studying effects of drugs upon behaviour. Some of these have seen but little use; others have become standard features in research laboratories. The range of behaviour patterns they generate is broad: food and water intake, general activity, sensory discrimination, maze learning, classical and operant conditioning, problem solving, timing behaviour, and many others. Those based upon principles of operant conditioning have been used with the greatest frequency, probably because they permit precise specification of the conditions for generating the behaviour to be measured, and because they can be adapted to generate a wide variety of behaviour patterns. Proper standardization of any of the test situations requires that: (a) its characteristics be described in full; (b) an exact procedure for its use be specified, including identification of all variables which might affect the behaviour pattern generated; (c) specification of the parameters measured and the methods of measurement; and, (d) information about the consistencies, or reliabilities, of the measures obtained (Carlton, 1963; Dews, 1962; Dews and Morse, 1961; Miller, 1956; Miller and Barry, 1960).

Acquisition, Maintenance and Extinction

With the exception of those which are considered to be primarily innate in origin, e.g., reflex action, behaviour patterns may be viewed in terms of the processes involved in their generation or acquisition, their maintenance once acquired, and usually, their extinction after the maintenance process itself has been examined. Drugs may affect any or all of these.

In past studies of the effects of drugs upon behaviour the maintenance aspect has received the greatest amount of attention, probably because this is the phase in which the behaviour pattern is the most stable, the least variable, and often the easiest to measure with precision. Practice may be continued until a stable level of performance, or behavioural baseline, is established for which the trial-to-trial variability of the measure of behaviour concerned is small and consistent. Effects of drugs can then be determined in terms of changes in the baseline. With proper precautions for carry-over effects, the same subjects may be used in dose-response studies, a very important consideration in view of the effort and time invested in generating the baseline in the first instance. Despite its advantages there also are limitations in using maintenance of behaviour as a measure of drug effects. Obviously the measure provides no information about how the behaviour pattern is acquired or extinguished, and in answering certain questions it is more important to know how drugs affect these processes. Behaviour patterns may be so overlearned as to be relatively insensitive to modification by drugs. This interaction between level of training and drug effects is nicely illustrated in results obtained by Steinberg, Rushton and Tinson (1961) which showed that, when rats were trained in a Y-maze to a point where a stable level of exploratory activity had been reached, small doses of an amphetamine-barbiturate mixture had very little effect; in contrast, animals without training showed very marked effects.

One of the major problems of studying acquisition and extinction lies in the fact that it is difficult to design a situation to generate acquisition and extinction that will permit a subject to be used more than once. When a subject is in the process of acquiring a behaviour pattern, he is different after each practice trial than he was at its beginning. Acquisition involves a change in behaviour and, therefore, provides no stable baseline with which to compare a subject's performance after administration of a drug. There is strong reason to believe that the differences may be reflected in all four basic properties of living organisms: anatomical, behavioural, biochemical and electrophysiological. Therefore, to face a subject again with the same behavioural situation involves carry-over, or what we call 'transfer of training.' Neither the same circumstances nor the same behavioural manifestations are present as those existing when the behavioural pattern was at an earlier stage in its acquisition. Comparisons must, therefore, be made between groups of subjects given different experimental treatments, i.e., appropriate control and drug groups, and interindividual differences in ability to learn become confounding variables. A subject cannot be used for repeated treatments except in such special situations as those involving serial problem solving or habit reversal, and here also there are problems of transfer effects which may confuse interpretation. The same kinds of difficulties arise in studying effects of drugs on extinction. The differential effects on behaviour which drugs may produce under different conditions of learning and drive level are clearly illustrated in results reported by Bindra and Mendelson (1963): chlorpromazine decreased the rate of a lever-pressing response after high levels of training but not after low levels; methylphenidate decreased the rate of the same response at low drive levels but not at high.

Studies of the kinds I have been describing show that drug-behaviour interactions are functions of basic parameters of the behaviour measured as well as of the various parameters of the drugs. The search for behavioural modes of action of drugs must take this fact into account. One of the advantages of research under controlled experimental conditions is that these potentially confounding effects can be identified and measured.

Measuring Instruments in Research on Human Subjects

Theoretically the problems of generating and measuring behaviour discussed above hold for all species, including man (e.g., Di Mascio *et al.,* 1963). However, practical considerations add important limitations. When research on human beings is involved, we run into a special problem facing all of our disciplines, namely, that it is difficult for us to expose human beings, for experimental purposes, to some of the kinds of behavioural situations—to generate the kinds of behaviour patterns—we might wish to study. It is equally difficult to use human beings as subjects at many stages in the study of the effects of drugs upon their behaviour or upon their neurophysiology or neurochemistry. For example, the obvious problems of administering drugs for experimental purposes have their counterparts in problems of exposing human subjects at least to certain kinds of behavioural experiences, e.g., to severe stress or to addiction. Such limitations prevent experimental studies of several aspects of behaviour in which we are particularly interested. Special procedures have, therefore, been worked out by psychologists for attempting to measure human behaviour in situations where the desired behaviour patterns already exist, e.g., in a clinical setting or in the individual's natural habitat where there is no question or ethical problem of establishing behaviour patterns solely for experimental purposes. It follows from what has already been said that much confusion and contradiction may arise from inadequacies in specifying the origins of such behaviours. This difficulty appears clearly in the literature describing effects of drugs on behaviour disorders classified in terms of the usual diagnostic categories. For example, 'schizophrenia' is a label for heterogeneous syndromes of multi-factorial origin; therefore, it is not surprising that the behaviour of all patients labelled as schizophrenic does not change in the same way after administration of a drug.

Despite problems arising from the lack of specific knowledge about the etiology of behaviour in the clinical setting, 'controlled clinical trials' have become the preferred method for the final evaluation of psychoactive drugs to be used in attempts to alter aberrant behaviour. Behaviour has been studied under two general sets of conditions. One of these has involved the use of symptom rating scales, behaviour inventories, and check lists as instruments to measure patient behaviour in a variety of 'natural', as contrasted with experimental laboratory, settings, e.g., in the hospital ward, at home, in the community. The development and standardization of these instruments has received considerable attention (Lorr, 1959); an 'Addiction Research Center Inventory' (Haertgen *et al.*, 1963) has been constructed specifically for use '. . . in differentiating various subjective effects of drugs and in discriminating some similarities and differences of naturally occurring and experimentally induced behavioural abnormalities.' The second set of conditions has centered around the use of psychological tests as measuring instruments, the test situation creating a temporary environment in which certain response patterns may be elicited and measured in as standardized a manner as possible.

In introducing the topic of behavioural modes of action of drugs I pointed out that attempts at this kind of analysis have tended to be oriented in three principal directions: (a) the comparative study of effects of different drugs on a single behavioural baseline, which, of course, may have several parameters to measure; (b) the comparative study of effects of one or more drugs on a battery of different behavioural baselines when the comparisons are made directly in terms of empirical measures of the behaviours observed; and (c) the study of drug effects on a battery of behavioural measures selected to provide information relevant to some theoretical construct, e.g., 'fear', 'memory', which may be part of a more general model of behaviour. Considerable information is available as a result of research of the first kind; less information of the second kind is now in hand, probably because of the considerably greater investment required to obtain it; relatively little of a systematic nature has come from the third approach, which involves the researcher in deliberate predictions from some theoretical model of behaviour.

QUANTITATIVE RELATIONS BETWEEN PARAMETERS OF A DRUG AND PARAMETERS OF THE BEHAVIOUR AFFECTED

In my introduction I referred to a second general question of particular interest from the behavioural point of view: What quantitative relations exist between parameters of a drug and parameters of the behaviour affected? The description of interactions between drugs and behaviour requires information about concomitant variations between parameters of each, e.g., information about dose-response and time-response relations, and about carry-over effects. Research using designs which have not provided such information earlier confused, rather than contributed to, the state of knowledge in psychopharmacology. I wish now to call attention to certain of these quantitative relations.

Dose-Response Relations

Kety (1961) has reasserted the view that drugs act as chemical stimuli, exerting their effects by modifying functions or processes which are already present in the organism. Viewed in this way, a drug, as any stimulus, may be described in terms of certain characteristics or parameters which determine the effects it produces in biological systems. Brodie (1962) has described the general nature of these characteristics as falling into two classes: (a) characteristics which permit a drug to reach its site of action in adequate concentration and (b) characteristics which determine the 'intrinsic activity' of the drug. Among the parameters of particular interest in the study of drug-behaviour interactions is its dose level.

In a sense, the dose parameter is analogous to the intensity dimension of auditory and visual stimuli, a dimension which in those sensory systems is related to such characteristics of behaviour as absolute and terminal threshold and to sensitivity within the range between them. Determination of dose-response relations may be used to provide analogous information about drug-behaviour systems.

Thresholds. In considering the specificity of the behavioural effects of a drug, we are concerned with two kinds of thresholds. The *absolute threshold* is defined as the minimum dose level which will produce the specific effect – the minimum effective dose; below this level the behaviour being measured is not affected. At the other end of the effective range is a *terminal threshold,* the maximal level of drug that produces the specific effects, beyond which non-specific effects interfere with behaviour generally. For example, in research on effects of anticholinesterase agents we have found no significant change in a variety of behaviour baselines until the dose level is sufficient to reduce the enzyme to about 60 percent of its normal activity; decrements in performance then continue to increase with increasing dose until the level of enzyme activity reaches about 10 percent of normal when convulsions and general motor impairment affect all forms of response.

Sensitivity between thresholds. The manner in which a specific behaviour measure changes with dose level between these thresholds provides the data for determination of dose-response functions. They also reveal changes in sign or direction. Plots of relations between dose level and a specific measure of behaviour are often monotonic. As reported in the research literature, behaviour shows impairment in many instances and enhancement in some. Other plots of the sensitivity of behavioural measures to drug effects indicate the existence of more complex relations. One common finding is apparent enhancement of performance followed by decrement. Research on the effects of anticholinesterase agents to which I referred earlier has repeatedly provided evidence for slight improvements in behaviour as the enzyme activity is lowered from its normal level, followed by rapid decreases in performance as the activity level is further decreased. Results from studies of d-amphetamine suggest that its effects on certain behavioural baselines may show similar trends.

Reference to studies of d-amphetamine allow me to make another point which I believe is particularly relevant here. It concerns whether or not many of the results reported using this and other drugs may in fact be interpretable in terms of non-specific, rather than specific effects of a drug upon behaviour. For example, the stimulating effects of d-amphetamine include increased general activity and motor movement; this non-specific effect could well account for enhancement of performance in test situations where increased general activity is an asset and for decrements in performance in situations where, for instance, the adequate behaviour pattern required that the subject inhibit or delay his responses. The same drug may stimulate or depress behaviour depending upon the nature of the particular response pattern selected for study.

Time-Response Relations

Another important parameter is the time-response characteristic of a behavioural baseline affected by drug administration. As with behavioural systems stimulated in other ways, if the dose level administered is within the effective range, the behavioural effects of a drug should be distributed over time as some function of the stimulus and of the state of the organism at the beginning of stimulation. Examination of typical plots of time-response characteristics shows that peak effects are often rapidly attained and are followed by more gradual recovery. There are exceptions to this observation. For example, in an experiment designed to study effects of reserpine on a variety of behaviour baselines affected, some reached their peak effect at two hours after drug administration and others not until after a longer delay; recovery of predrug levels of performance occurred within 10 to 24 hours. The determination of the two time parameters, peak effect time and duration of effect, is important in specifying the characteristics of drug-behaviour interactions and, as I will point out later, is essential in searching for neurochemical or neurophysiological mechanisms that may be related to the behaviour.

Variability

Another major characteristic of drug-behaviour interactions which can be analyzed quantitatively is their variability. In much of the literature in psychopharmacology, there is relatively little attention given to this parameter of behaviour. Yet drug effects may appear mainly as changes in the variability of the behavioural baseline. There are examples in which the mean level of the response being measured remained essentially unchanged after administration of a drug, yet the variability in performance of subjects affected increased very significantly. Two different types of variability need to be examined as a regular feature of research on drug-behaviour interactions.

1. *Intraindividual variability.* Individuals characteristically vary in their performance from trial to trial, even when the conditions generating the behaviour are held as objectively constant as possible. Such intraindividual variability tends to decrease as the behaviour pattern is learned and overlearned. In order to study this kind of variability, it is necessary to have more than one measure of performance during each test session. Whether this is possible depends, of course, upon the nature of the behaviour measured and, therefore, upon the conditions under which it is generated. The several measures taken during a test session constitute a distribution of measures from which standard statistics of central tendency and variability may be calculated. Our usual procedure is to compute a standard deviation, s, for each subject at the peak effect time following each drug administration. The standard deviation then constitutes a 'score' for the subject's variability of performance and can be compared with his analogous scores under other treatment conditions or with scores obtained by other subjects. The significance of observed differences is tested by analysis of variance procedures.

2. *Interindividual variability.* Characteristically, individuals differ from each other in the levels at which they perform the same task, even when intraindividual differences are minimized. It is possible that one effect of chemical stimulation is an alteration in such interindividual differences. The effect would appear as changes in the spread of the response measures for individual subjects during the drug period as indicated, for example, by the magnitude of the standard deviation at each dose level.

The two kinds of variability discussed above have been found by behavioural scientists to be of particular significance in other contexts. Much fuller examinations should be made of these quantitative parameters of behaviour. It is quite possible that drugs may affect the variability of behaviour without causing any striking change in its central tendency. I believe that the study of such variability may be particularly important in research on problems of addiction.

Carry-Over Effects

One pervasive characteristic of the behaviour is the carry-over effect that occurs when a response pattern is repeatedly elicited; this is another area of quantifiable problems. Transfer of effects from one experience to another is the rule, certainly not the exception, when behavioural variables are being observed. The acquisition phase in the learning process is a prime example: changes occur on one trial which are functions of modifications of responding induced during previous trials. Carry-over effects may also occur in studies of drug-behaviour interaction. Analyses for such effects in our studies have suggested that three different processes may be involved.

One such process can easily be observed in situations involving the repeated administration of a drug to the same subject. It is dependent upon the continuing presence in the body of one dose when another dose is administered. Pharmacologically, a drug is considered to have a cumulative action when its elimination and/or catabolism are relatively slow and the full response is the result of the summation of two or more doses.

Cumulative effects may also occur when the after-effects of one dose of a drug on a neurochemical or neurological event still persist in the body at the time another dose is administered, even though drug from the first dose is no longer present. In this instance carry-over effects result from overlapping responses of the neurochemical system to chemical stimulation. In my opinion, the processes of addiction provide some clear examples of carry-over effects of this kind.

In a third instance, carry-over effects may result from the mere procedure of repeated measurements of the same behaviour pattern. It is also possible that performance after administration of a drug may produce special behavioural effects which persist after the drug has been completely eliminated or metabolized. An experiment by Behar and Riopelle (1957) illustrates this possibility by demonstrating a persistant retardation in the acquisition of a conditioned avoidance response following a period of trials under the drug, reserpine; the authors suggest that '. . . some new adaptive response seems to be acquired during the drug phase which persists into (the post drug trials)'.

In my opinion too little attention has been given to the importance of carry-over effects in studies involving the repeated use of the same subjects. Carry-over effects are often a nuisance! Economy of time and effort and the nature of the hypothesis under test make such repeated use desirable, but the possibility of resulting carry-over effects may introduce complications in the interpretation of results. The usual precaution has been to provide intervals between administrations of sufficient length presumably to eliminate the possibility. It is difficult to set a proper interval unless the time characteristics for elimination of the drug and for recovery of biochemical systems are known.

In some of our earlier studies we tried the approach of conducting preliminary experiments to determine the time required for the behaviour being measured to return to predrug baseline levels after an acute administration of the maximum dose we intended to use in the main study. We added a safety margin to this recovery time. Even then we were fooled, for later checks showed that carry-over had taken place despite our precautions. It appeared that we had not given proper attention to the threshold phenomenon described earlier; the neurochemical system stimulated by the drug had recovered sufficiently to be subthreshold for the behaviour but had not recovered completely, thus producing an additive effect when repeated administrations were involved.

Another procedure has been to plan the research in such a way that the presence of carry-over effects can be determined. Latin square and factorial designs have been used for this purpose. Unfortunately, if carry-over effects are found to occur, it may be very difficult to identify their source.

Dose-Order Effects

Since dose level is such an important parameter of drugs used in chemical stimulation of biochemical systems, it might be expected that any carry-over effects during repeated stimulation would be some function of the sequence or order in which various doses were administered. For example, it might be predicted that higher doses given early in a sequence would show greater carry-over effects than lower doses given early.

By using a Latin square research design involving replication of the same square it has been possible to isolate a sum of squares corresponding to the particular orders of administration of the various doses employed. This ‘orders’ sum of squares could then be used to test whether the sequence of administration did, in fact, produce significant effects upon the behaviour patterns measured. I can only report at this time that we have never found a significant dose-order effect.

NEUROCHEMICAL CORRELATES OF BEHAVIOUR

The third general question I raised earlier had to do with *neurochemical and neurophysiological correlates of behaviour.*

In my discussion so far I have been concerned primarily with hypotheses in which drugs serve as independent variables, and behaviour as dependent variables. There still remains the task of ‘filling the empty organism' (Russell, 1961), of considering effects of drugs on interactions between behaviour and other properties of the organism, e.g.

$$\begin{tikzcd}\text{Drugs} \arrow[r] & \begin{array}{c}\text{Neurochemical}\\ \text{Events}\end{array} \arrow[r, shift left, "1"] & \text{Behaviour} \arrow[l, shift left, "2"]\end{tikzcd}$$

In this area of problems we find some of the most exciting theoretical and research possibilities in modern psychophysiology. You will recall my statement that it would appear from the evidence available so far that relations between behaviour and these variables are reciprocal processes in which changes in neurochemical events may affect behaviour and changes of behaviour may affect neurochemical events. Such interactions introduce complications in research in psychopharmacology because we are measuring the effects of drugs upon behaviour and in the process of taking the measure of behaviour we may be altering biochemical events upon which the drugs have their actions. Our subjects become different organisms neurochemically, neurophysiologically, and psychologically as the behavioural testing goes on. Psychologists, and colleagues interested in basic behavioural events, are looking for neurochemical correlates of behaviour at three levels: (a) the nucleic acid macromolecules, (b) the enzymes produced by protein synthesis, and (c) neural growth factors which are also protein in nature.

Problems of Research Design

To establish the fact that certain measures of behaviour are indeed related to certain neurochemical events within the organism requires evidence that the two vary concomitantly. Such evidence is based upon empirically determined time characteristics of the behavioural measures and of the neurochemical events with which they are alleged to be related. Good research design requires that the independent variable, a particular neurochemical event, be varied systematically while some standardized measure(s) of behaviour, the dependent variable(s), is measured. If the latter varies as some function of the former, the two may be said to be related—*but* only if other variables, which might affect the behaviour baseline being measured, are controlled. It is in meeting these requirements of research design that significant problems arise.

What neurochemical events should be selected as independent variables and by what means can they be varied systematically? The research literature indicates that the alleged neurohumoral transmitter systems have received the major attention as the independent variables of choice, the acetylcholine (e.g., Carlton, 1963; Fisher and Coury, 1962; Grossman, 1962a, 1964; Russell *et al.,* 1961; Stein, 1963, Stein & Seifter, 1962), norepinephrine (e.g., Grossman, 1962b), and serotonin (e.g., Aprison and Ferster, 1960, 1961a, 1961b) systems being the favorites to date. Next in order have come events associated with protein synthesis (e.g., Cook *et al.,* 1963; Dingman and Sporn, 1961; Flexner *et al.,* 1963). Drugs have been used to alter these events.

What variables should be controlled? It is clear from the earlier discussion that variables which may interact with the behaviour being measured in experiments of the kind we are considering could come from the test situation in which the behaviour is being generated, from side effects of the drugs being used as tools to vary the neurochemical events under study, or from characteristics of the behavioural baselines themselves, e.g., drive level, level of training. In many instances not all the relevant variables have been fully identified; even where they are known, control of them may still be difficult.

What behavioural variables should be selected and how can they be measured? These questions have already received as much attention as we can give them in this paper.

To achieve completely satisfactory research designs in this area at this time may be more a hope than a possibility for achievement. Many of the problems are a result of the lack of sufficient information in the disciplines whose collaborative efforts are essential to the search for neurochemical correlates of behaviour. The search is now in the stage when advances must be made by successive approximations, eliminating errors and sharpening tools as research progresses.

Effects of Behaviour on Neurochemical Events

At several points in the discussion I have referred to evidence which suggests that relations between neurochemical events and behaviour are reciprocal in the sense that changes in one may affect the state of the other. So far attention has been focused upon situations in which neurochemical events are the independent variables and behaviour, the dependent. The evidence that these roles may be reversed comes from several sources (e.g., Chitty, 1955; Clarke, 1953; Krech *et al.,* 1960; Hydén, 1961; Hydén & Egyházi, 1962). The research results reported in all these studies indicate that persisting changes in biochemical events may be induced by an organism's behavioural reactions to its external environment: changes in behaviour precede concomitant changes in biochemical events.

The implications of a reciprocal interrelationship between neurochemical events and behaviour deserve very careful exploration. One issue is clearly apparent: If behavioural events produce changes in neurochemical systems, then, as behaviour varies, so will the neurochemical substrate upon which drugs may act; this, in turn, may influence the nature of drug effects upon behaviour.

SOME ADDITIONAL AREAS FOR RESEARCH

There are certain additional areas of research which are closely related to those already considered and which are of particular interest to those concerned with the behavioural aspects of neuropharmacology. I wish briefly to introduce some of them before ending this paper.

Site of Action

In discussing the biochemical and neurophysiological action of psychoactive drugs, Dr. Himwich (see Uhr & Miller, 1960) once stated: 'It is well known that every drug may affect many parts of the body, but it must also be emphasized that some areas, usually the important ones for the therapeutic results, are more susceptible than others.' The research literature has shown an increasing interest in discovery of such sites of action and in relating neurochemical events in them to behaviour; the search becomes directed toward relations between effects upon behaviour which result from neurochemical events occurring in particular structural locales. Distinctions between central and peripheral effects have been attempted by comparing measures of behaviour when the same drug is administered peripherally through one of the usual routes and centrally through cannulae into the ventricular system. Use has also been made of the fact that certain drugs pass from peripheral blood into brain more readily when they are in one form than another, e.g., atropine and methyl atropine. More precise localization has been achieved by implantation of microcannulae into particular sites of interest. Research by Grossman (1962a, 1962b) has become a favorite example of the search for sites where specific behaviour patterns may be 'neurochemically coded': a statement from one of his 1962 papers makes the point: '. . . the placement of adrenergic substances into a circumscribed region of the diencephalon elicits specific changes in one type of motivated behaviour (food intake) while the placement of cholinergic agents into the same area of the hypothalamus evokes pronounced changes in a different type of motivated activity (water intake).'

Neural Growth Factors

During my earlier discussion I referred to two levels of neurochemical events which have received most attention in the search for neurochemical correlates of behaviour. A third level deserves brief mention because of its intriguing possibilities as a means of accounting for such persistent characteristics of behaviour as long-term memory. I refer to the demonstrations by Levi-Montalcini and her co-workers of the presence of growth-promoting substances, neural growth factors, in a wide range of tissues and body fluids and in a variety of animal species, including man. These substances, protein in nature, are highly specific for nervous tissue. The sympathetic nerve cells of birds and mammals are particularly receptive to one of them; the same protein has been found to be a normal constituent of sympathetic cells. One of the interesting phases of the research has been the preparation of an antiserum which selectively affects the sympathetic neurones of new born animals without affecting other nerve cells or other tissues, leaving experimental animals comparable in other ways to normal controls. The interesting possibility suggested by the research is that interactions may occur between behaviour and the neurochemical events involved in the synthesis of NGFs which could modify the structure of nerve cells and, thus, have a long-term effect upon behaviour.

Neurochemical Event—Behaviour Systems

There are two general points under this heading which deserve some special attention. The first involves a methodological issue; the second concerns the need for more complete research analyses of the characteristics of specific neurochemical event-behaviour systems once they have been identified.

In the present and past literature there has arisen a methodological issue relating to the identification of what I will call, for shorthand purposes, 'neurochemical event-behaviour systems.' Earlier I pointed out that to establish the fact that certain measures of behaviour are indeed related to certain neurochemical events within the organism requires evidence that the two vary concomitantly. To be fully convincing, the data upon which such evidence can be based should be acquired by measuring changes in both neurochemical events and behaviour under the same experimental conditions and comparing their time characteristics for evidence of concomitant variation. This has been done in only a few instances (e.g., Aprison and Ferster, 1961a, 1961b). In many more instances the argument has been based upon the fact that, in one set of experiments, a particular drug has been found to produce changes in a neurochemical event and, in another set of experiments, that the drug has produced changes in behaviour; therefore, it is concluded, the neurochemical event and the behaviour are related. In view of the many relevant variables which may have been operating in different ways in the two sets of experiments, it is obviously dangerous to reach conclusions about neurochemical event-behaviour interactions in this way. Such evidence may be fruitful in suggesting hypotheses about possible interactions, but the hypotheses must then be tested by experiments designed specifically for the purpose. I have raised this issue because it is inherent in a number of papers in the recent literature.

My second point under the present heading is intended to call attention to the need for research which is designed to analyze fully the characteristics of a neurochemical event-behaviour system once it has been identified. For example, the research by Grossman and others discussed briefly above indicates that the regulation of water intake may be controlled by direct cholinergic stimulation at circumscribed areas in the hypothalamus. This suggests that neurochemical events in these areas may be involved in the construct of 'thirst' as a primary drive. If so, direct stimulation of these events could serve to motivate the acquisition of new behaviour patterns reinforced by water reward and might also be reflected in the retention and extinction of the new patterns once they were established. We have completed several experiments to test these hypotheses by comparing effects of direct cholinergic stimulation with effects of conventional water deprivation. The results show that analogous effects on consummatory behaviour, on the acquisition of new behaviour patterns, on the relearning of the patterns following a period of no practice, and on the maintenance of performance once the patterns had been learned can be produced by the two quite different conditions. This illustration is intended to support the plea for more complete analyses of the characteristics of neurochemical event-behaviour systems once they have been identified.

Administration of Drugs during Early Development

Another particularly interesting area for research centers upon effects on behaviour which may result from the administration of drugs during an organism's early development. The thalidomide story raised considerable concern about interactions between drug effects and the development of anatomical structures. There have appeared a number of experiments which indicate that the administration of drugs to the pregnant mother or to the neonate during the early days after birth may affect such postnatal behaviour as 'emotionality' and learning (e.g. Young, 1963, 1964). This area of research in behavioural neuropharmacology deserves to be explored much more fully than it has been, not only because of its practical implications but also because of the contributions it can make to our understanding of drug-neurochemical event-behaviour interactions.

A FINAL NOTE

This paper began with a rather strong statement about the present difficulty in drawing general conclusions about the effects of drugs upon behaviour. Emphasis was intended to be upon the adjective 'general.' The literature in the field is large and has been growing rapidly during the past decade. Research methodology has also been in the process of becoming more sophisticated; ideas and concepts have been formulated, examined and revised. All these are healthy signs of interest in the problems of behavioural neuropharmacology.

One of the major issues for those concerned with the behavioural aspects of this interdisciplinary field has been the ways in which basic questions about relations between behaviour and the other relevant variables can most profitably be formulated. As is generally the case in newly developing fields, it is easy to become heavily involved in very specific problems, to the exclusion of a broader view of the gestalt of which the specifics are a part. I have chosen to concern myself with what I consider to be basic questions about the behavioural aspects of the general topic we are considering at this conference and about the relations which may exist between these and other aspects of the topic. I believe that from the broader view are more likely to come suggestions about what aspects of the general topic are most relevant and most fruitful for investigation now and in the immediate future.

DISCUSSION OF DR. RUSSELL'S PAPER

Dr. Russell: Having dealt with the more general points to be considered under the heading of 'Behavioural Effects of Psychoactive Drugs', I now wish to try to illustrate some of these points by looking at a *particular* area of problems involving relations between drugs and behaviour, the area that we have met to discuss. I wish to examine the topic of addiction and, to keep the examination reasonably short, I will concern myself only with narcotic addiction.

Systems Approach to Study of Addictive Behaviour

Addictive behaviour: How is it acquired? How is it maintained; How is it extinguished? The questions have been put in other ways, but these are in terms that are most meaningful to behavioural scientists. To consider the topic in these terms not only illustrates the study of acquisition, maintenance, and extinction of behaviour patterns under drug conditions, but also provides an opportunity to emphasize an approach which I think is particularly important in research on the general issues we are considering at this meeting. That is, the importance of what has been called a *systems approach* (Weeks, 1964) in contrast to an approach dealing solely with extreme positions, solely with relations between drugs and single properties of living organisms (Wikler, 1953). It seems essential for us, in our overall view, to consider the living organism as a system, with drugs affecting inter-relations between all of the properties of that system. Indeed, when we become involved in considerations of the behavioural aspects of drug addiction, we find ourselves involved in examining environmental conditions—social and cultural factors—affecting the process of acquiring, maintaining and extinguishing the addiction behaviour, as well as with biochemical and physiological characteristics of the addict himself (Small *et al.,* 1938).

FACTORS INVOLVED IN ACQUISITION OF ADDICTIVE BEHAVIOUR

Several times I have suggested that, from a behavioural point of view, we can look at the development of addictive behaviour as an example of the effects of drugs upon the processes of acquisition, maintenance, and extinction of the behaviour pattern. The major questions to be asked, in the study of addictive behaviour, really fall into these three general categories. First, then, 'How is the behaviour acquired in the first instance?' What are the basic factors that are involved in the development of the behaviour pattern? When I speak of addictive behaviour, I of course recognize that there may be a very considerable range of interindividual differences in the specific responses made. The specific responses may have certain very general features in common for all addicts, but in their details they are not stereotyped—not of a push-button or slot-machine nature. In examining the question, we must look, first of all, for the basic means by which such a variety of responses can be reinforced. In its early stages the response of 'experimenting' with drugs must be supported in some way in terms of the idiosyncratic motivation of the individual concerned and of the kinds of satisfactions to this motivation that result from drug taking. From suggestions in the clinical literature, there appear to be three general possibilities here, which we may describe in terms of *primary drives, secondary drives* and *imitation.*

Basic Drive Stimuli

First of all, there is a possibility that the behaviour pattern involving experimenting with drugs may be initiated by certain basic *drive stimuli* which are dependent upon biological characteristics of the organism and are innately determined. By drive stimuli, I am referring to changes of tissue conditions within the body that are associated behaviourally with an increase in activity, and eventually with the making of a response which is instrumental to returning the basic tissue conditions to their resting or normal state. A relatively simple paradigm is to be found in food-seeking behaviour.

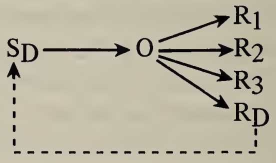
One of the traditional techniques employed in studying the acquisition of new behavioural patterns involves depriving experimental subjects of food and, after a period of time, using food rewards to reinforce the particular behaviour to be learned. Behaviourally, the effect of food deprivation is to increase the subject's general activity and to maintain the increase until the consummatory response of food intake occurs. Responses which are *instrumental* to obtaining food are reinforced and the probability of their reoccurrence in the future is increased; other responses are extinguished. Following food intake, general activity decreases and the instrumentally-conditioned responses which led to food are no longer elicited. Clearly, the deprivation conditions lead to some change in internal tissue conditions which, in turn, initiate the behavioural changes I have just described. A very considerable amount of research has been carried out in an effort to describe the nature of these tissue conditions (see summary in Morgan, 1965). We know that a functional neural circuit exists, involving several interrelated limbic and diencephalic structures, which is selectively sensitive to direct chemical stimulation of a specific nature, i.e., to adrenergic stimulation (Grossman, 1962). The neurochemical coding is also capable of carrying quantitative information in such a way that the magnitude of the consummatory response is related to the extent of deprivation.

This is an example of what the psychologist refers to as a *primary motivational system*: changes in tissue conditions within the body set up drive stimuli, S_D, eventuating in a response which returns the tissue conditions to their normal steady or resting state. It is a kind of behavioural homeostatic mechanism. For our present purposes, the most important point is that the mechanism has the capability of incorporating instrumentally-conditioned responses, of supporting the acquisition of new behaviour patterns. Some such sequence of events may be involved in the acquisition of addictive behaviour.

Dr. Mary Brazier: What parallel would there be for that in man?

Dr. Russell: Primary or basic drive systems of the kind I have been discussing are found in all animal species, including man. Indeed these systems—hunger, thirst, pain—are just as effective in controlling behaviour in man as they are in simpler organisms. The situation becomes much more complicated by the fact that, on the basis of these primary drives, an extensive system of *secondary drives or motives* are acquired. I will discuss these later, but I wish now to emphasize the contributions which *primary* drives can make to the acquisition of addictive behaviour and to its maintenance once it has been learned.

The suggestion has been made by Beach (1957a), Wikler (1962) and others that the basic motivation involved, the S_D or drive stimuli, in this instance may depend primarily upon tissue conditions that are associated with physical discomfort or pain. One possibility, particularly in the case of young adolescents, is that changes in secondary sex characteristics, the increase in sex drive, give rise to frustration and discomfort under cultural limitations, and that this leads the individual to seek various responses which will reduce the tensions. In the particular instance of drug-taking behaviour, one of the possibilities is that these tissue conditions set up drive stimuli, S_D, which activate the individual generally.

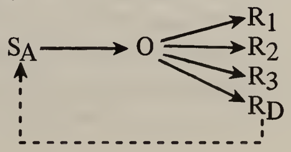


Among other responses is that of experimenting with drugs, R_D, including narcotics, which have the unique ability to reduce primary drives and notably those that are related to pain (Wikler, 1962). The reduction of basic drive stimuli then reinforces that particular response, increasing the probability of its occurrence in the future. Other responses that are not so reinforced drop out, are extinguished. So one possibility of accounting for the early acquisition of drug-taking behaviour could be dependent upon the effects of primary drives of this kind due to changes in tissue conditions.

Acquired Drives

A somewhat more complicated and sophisticated situation is dependent upon what N. E. Miller (1959) and others have referred to as ‘fear’ or ‘anxiety reduction.’ ‘Anxiety’ in this case is a *secondary or acquired drive.* A very simple example of the process involved in the development of such drives is the behaviour of experimental animals in what is now referred to as the classical conditioned avoidance situation, a situation which has become quite standard in experimental psychopharmacology. The conditioned avoidance response is acquired in a simple shuttlebox consisting of two compartments with grid floors through which mild electric shock can be administered. The subject is placed in one compartment and a light stimulus is presented, followed a few seconds later by activation of the grid. The subject can escape the shock by moving into the second compartment, where the grid is not active. As trials go on, the subject responds with shorter and shorter latencies to the administration of the shock and soon learns to avoid the noxious stimulus altogether by responding when the light stimulus appears and before the grid is activated. For us at this time, the most important characteristic of the situation is that the light stimulus, which originally did not have the motivational properties of the shock, now, through association of the two, has acquired those properties. This is evidenced when the subject is required to learn a new instrumental response, e.g., opening a door in order to move to the second compartment, with only the presence of the light stimulus to motivate its behaviour. Presumably this previously neutral stimulus possesses its motivation properties because it produces drive stimuli, S_D, which are anticipatory of some physical discomfort or pain, and which are encompassed by the theoretical construct of ‘fear’ or ‘anxiety.’ Such secondary or acquired drives are prominent features of behaviour in man, as well as in other organisms, and are important concepts in clinical as well as experimental analyses of behaviour.

It is possible, then, to think of addiction behaviour as being acquired in the first instance, not by reduction of a primary drive, but rather in terms of secondary drive or anxiety reduction. The general diagram would be quite similar in its characteristics to the one I used in describing the mechanism of primary drives, but in this instance the stimuli, S_A, that activate the behaviour are stimuli that have been associated with primary drives in the past and have acquired certain properties of the latter.



In this instance, a response R_D, which leads to reduction of the anxiety would be a response which would be reinforced and, therefore, would tend to be made again in the future. The taking of narcotics, experimenting with drugs, is one way in which anxiety-reduction can be achieved temporarily.

Dr. Domino: Dr. Russell, when you refer to narcotics, I gather that you are not really saying narcotic analgesics, but any drugs that act on the nervous system.

Dr. Russell: A number of drugs act in this way.

Dr. Domino: You would include barbiturates as well as narcotic analgesics?

Dr. Russell: Yes. The mechanisms I am describing now might very well be involved when alcohol, amphetamine, LSD or other drugs are involved. I want to try to be a bit more specific by just using narcotics as one example at this time.

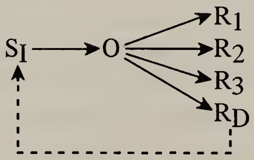
Dr. Domino: What about boredom? Do you think boredom could be an important drive to initiate some forms of experimentation with drug-taking? I wonder if this would not be an additional item in the category of secondary drive. You talked about anxiety reduction; I wonder if we could postulate that boredom reduction would be equally important.

Curiosity

Dr. Russell: I am not quite sure I know what you mean by boredom, but certainly it is possible to motivate behaviour in man and in other animals by exposing them to conditions of minimum or constant environmental stimulation. 'Curiosity' is a term which has recently been introduced in describing a wide variety of responses seemingly learned without involvement of any primary or obvious secondary drives (Berlyne, 1960). Experimental animals, whose primary drives have been satisfied, when placed in a new environment will spontaneously explore it. They will explore more thoroughly those situations in which they encounter numerous and complex stimuli, than simpler environments. They will learn responses which are instrumental to producing changes in their environments. Many of the motivational properties of primary drives appear to be present. We still know very little about the mechanisms underlying 'curiosity,' but its behavioural features have been extensively explored.

Imitation

Now another possibility that is, I think, in a different class than the two we have talked about already, is one that might be referred to as 'imitation.' To define such a concept, we have to be able to specify the conditions under which the behaviour is generated, and the nature of the behaviour once it appears. By 'imitation' I refer to the observation that a great many secondary motives influencing the behaviour of human beings arise from social interactions and these social interactions tend to produce behaviour in conformity with the behaviour of the group. The individual is reinforced by the approval of the group for behaving in particular ways. Such approval is a secondary or acquired incentive, a part of the secondary drive system, and is a particularly potent one. When we think of how hard all of us work for incentives of this kind, we cannot but think of them as perhaps the most important of the factors that motivate our more complex behaviour patterns. Stimuli of a social nature may also serve to initiate drug-taking responses, which then lead to the reinforcement in the form of social approval. The circumstances are basically similar to those pertaining to anxiety-reduction. In both instances secondary or acquired motivational systems are involved. Efforts to gain social approval lead to many different responses; experimenting with drugs, if it leads to social approval, is reinforced and, hence, its probability of appearing again is enhanced.



Genetic, Social and Cultural Factors

Before we leave the acquisition phase of addiction behaviour I wish to point out that there are factors which may significantly influence the acquisition process and which, therefore, may be of special interest to us in our later discussion. These may be classified within the broad categories of (a) genetic factors and (b) social and cultural factors. Clearly, individuals differ in their responses to the three sets of conditions I have just discussed: the majority respond in ways other than the acquisition of drug-taking behaviour, and addiction itself is characterized by a wide variety of specific behaviour patterns. These individual differences are certainly influenced by experience and learning which take place prior to the occurrence of conditions favourable to the acquisition of addictive behaviour; much of the significant learning and experience, probably the most important part of it, occurs within the individual's social and cultural setting. In my opinion, an understanding of the totality of events contributing to the acquisition of drug-taking behaviour requires that as careful attention be given to anthropological and sociological research as to research on the biological and psychological factors involved (see, for example, Lindesmith, 1947). It is also important to consider the effects which genetic differences may have upon the acquisition process, for we know that they have significant effects upon the learning of other behaviour patterns. The point I wish to emphasize is that, in taking a systems approach to the study of addiction, we must be concerned with factors which have affected the individual in the past and which may have carried over to influence his present responses under circumstances where the acquisition of addictive behaviour is a possible outcome.

FACTORS INVOLVED IN MAINTENANCE OF ADDICTIVE BEHAVIOUR

Primary and/or Secondary Drives

Now let me turn to the *maintenance* phase of the behaviour once it has been established. Here I think we must introduce a new condition into the behavioural analysis; emphasis must now be placed particularly upon neurochemical and neurophysiological factors. The empirical observation is that, once the behaviour has been acquired in the first instance, it is vigorously maintained and is extremely difficult to extinguish. The question arises as to whether the maintenance of the behaviour under these circumstances continues to involve *only* the primary and/or secondary motivational systems that I described earlier in relation to acquisition. Certainly they may continue to operate, but the circumstances in which they operate may change greatly: in continuing the addictive behaviour, the addict may subject himself to all sorts of social and general environmental circumstances that would have been unfavourable to the original acquisition, and yet the behaviour persists. This resistance to extinction, even in spite of deliberate therapeutic efforts to alter the conditions which supported acquisition, suggests the probability that some new primary drive(s) has been established.

Neurochemical and Neurophysiological Changes in Drive Stimuli

The appearance of the phenomena of 'tolerance' and of 'physical dependence' indicate that changes of a neurochemical and neurophysiological nature occur as the drug-taking behaviour continues. The possibility here is that, because of the pharmacological effects of the drug, there has occurred a change in tissue conditions and that the new tissue conditions themselves serve in the manner of a primary drive to motivate the maintenance of the behaviour once it has been established. In order to reduce the tissue conditions that have been produced by the drug, it is necessary to take the drug again. There is, presumably, some alteration of a normal steady state system within the body that persists and gives rise to 'withdrawal symptoms' when the drug is withheld. We may diagram this basis for the drug-taking behaviour as follows:

$$\begin{tikzcd} S_D{}^1 \arrow[r] & O \arrow[r] & R_D \arrow[ll, dashed, bend left] \end{tikzcd}$$

In this case $S_D{}^1$ represents drive stimuli arising from the new tissue conditions, produced by the earlier intake of the drug itself. As long as these drive stimuli persist, the individual can be expected to continue the drug-taking response, which he has learned will lead to their reduction. Understanding the nature of the neurochemical and neurophysiological changes produced by the drug—their site, mode of action, and relations to parameters of the drug—is very important. It is a key issue in understanding how the addictive behaviour can be maintained, once it has been established in the first instance. There have been a number of suggestions as to what the basic nature of these changes may be: changes in steady states, reactions in enzyme systems, recruitment of auxiliary neural pathways not normally active in a functional sense, augmentation of irritability in certain cells of normally functioning systems, degenerative cellular changes (see, for example, Wikler, 1962; Corssen and Skora, 1964).

FACTORS INVOLVED IN EXTINCTION PHASE

Removal of Reinforcing Drug

Response Substitute

The final phase of this analysis is concerned with what behavioural scientists call 'extinction'—'withdrawal' in the terminology of addiction research. Extinction of behavioural patterns is produced by removing the reinforcer. In addictive behaviour extinction would be brought about by eliminating drug intake, which is the reinforcer maintaining the behaviour. If we could generalize from other forms of behaviour, we would predict that under extinction circumstances the drug-taking response would disappear. One important parameter would be the nature of this extinction process as a function of time. We would predict that other responses would be attempted which might substitute for the drug-taking response as possible means of reducing the basic tissue conditions involved in the maintenance of the behaviour. We would also predict that, if the organism were unable to find a suitable substitute response, there might appear what is often referred to as 'displacement activity', behaviour patterns which are not useful to the individual in adjusting to the extinction conditions. These general principles have been demonstrated many times in studies of extinction involving other forms of behaviour.

Displacement Activity

All these types of reactions to withdrawal or extinction have been reported in the clinical literature on addiction. In narcotic addiction these reactions are accompanied by responses characteristic of stimulation of certain functions under control of the sympathetic nervous system – e.g., sweating, increased secretions and motility causing vomiting and diarrhea, increased blood sugar from increased secretion of adrenalin, raised blood pressure (Kolb and Himmelsback, 1938) – which have implications as to the site of the changes in tissue conditions induced by the drug. In any event, the withdrawal or extinction effects, if dependent upon the altered tissue conditions, would be expected to continue until these conditions had returned to their normal, pre-drug steady state. This is another form of carry-over to which I referred in my formal paper: the persistence of after-effects of a drug on a neurochemical system after the drug itself is no longer present in the body.

Carry-over Effects at Behavioural Level Due to Conditioning

There is another kind of carry-over effect which I think is particularly important to our discussion. It seems to me that during the extinction of addiction behaviour, we might very well expect great difficulty to result from carry-over effects at the behavioural level. What I mean by this is that the drug-taking response that I referred to as R_D in my earlier examples might very well become conditioned in the classical sense to exteroceptive stimuli during the maintenance period. That is, conditioning may occur because certain exteroceptive stimuli are associated quite regularly with the drug-taking response. These stimuli, when they occur during the extinction period, would tend to reinforce the drug-taking response even further and, therefore, themselves have to be extinguished. Difficulty in extinction or withdrawal would then be dependent not just upon the return of neurochemical-neurophysiological processes to their normal steady states, but also upon behavioural carry-over effects due to conditioning of the kind I have just described. Not only might there be carryover effects due to classical conditioning with exteroceptive stimuli, but also with interoceptive stimuli as well. Interoceptive stimuli that are akin to those generated by the visceral disturbances associated with the withdrawal symptoms might then, when they occurred for other reasons, also tend to elicit the drug-taking response and thus complicate the whole process of extinction.

My objective in this general discussion has been to supplement the formal paper submitted for your consideration prior to the symposium, by examining addictive behaviour within the context of principles derived from the psychology of learning and motivation and from the relatively newer field of psychopharmacology. In applying these principles for research purposes, investigators have, for reasons very familiar to us all, chosen to follow a comparative approach in which much basic work is being done on infrahuman animal subjects. The difficulties in extrapolating from such animals to man, and even from species to species, are also well known (Russell, 1964b). But the comparative approach has paid off so handsomely in medical research as to reinforce its use in attacks upon the basic problems of addiction, including those of a psychological nature.

Models Used for Study of Addictive Behaviour

Research workers have demonstrated that addictive behaviour can be induced experimentally in infrahuman animal subjects, e.g., in rats, monkeys and chimpanzees (Beach, 1957a; Seevers, 1936; Spragg, 1940; Weeks, 1964). Two principal techniques have been employed: (a) those involving operant conditioning, such as escape training (Nichols and Davis, 1956; Nichols *et al.*, 1956; Thompson and Schuster, 1964), and (b) those beginning with forced administration of the drug (Weeks, 1962). Efforts have been made to determine the specific roles of reinforcement in the acquisition process (Beach, 1957a; Davis and Nichols, 1962; Lindesmith, 1946; Thompson and Schuster, 1964; Wikler, 1962). Repeated administrations of the drug have been reported to produce the phenomena of tolerance and physical dependence (Seevers and Deneau, 1963; Weeks, 1962, 1964). Evidence has been presented that these phenomena are associated with homeostatic changes or imbalances (Nichols, 1965; Seevers and Deneau, 1963; Thompson and Schuster, 1964; Weeks, 1962; Wikler, 1962). 'Abstinence' syndromes have been described as occurring under conditions of extinction or withdrawal (Beach, 1957b; Nichols, 1965; Thompson and Schuster, 1964; Weeks, 1964). And some attention has been given to factors which may affect addictive behaviour during its acquisition, maintenance and extinction phases (Davis and Nichols, 1962; Lindesmith, 1946; Nichols and Davis, 1956; Nichols and Evans, 1963; Seevers and Deneau, 1963). These, and other similar investigations, are leading to the development of a theoretical model of addictive behaviour which will integrate the facts already known, from which more precise predictions can be made for empirical testing, and from which applications of a clinical nature may be derived.

Comparative pharmacology of some psychosedatives

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INTRODUCTION

It is appropriate that this conference consider the comparative pharmacology of ethyl alcohol, barbiturates, and other substances used to produce mild sedation or tranquillization in man. Few have critically and systematically compared in detail the biochemical, physiological, and psychological effects of very old drugs such as ethyl alcohol with agents made available in the last 60 years such as the barbiturates, nonbarbiturate sedatives, and the newer tranquillizing drugs with antianxiety and/or antipsychotic properties. Inasmuch as all of these substances calm or sedate psychic processes, the more general term, psychosedative, has been used to describe these drugs.

Several investigators have tried to synthesize the divergent data obtained from many animal species, including man, as to where such drugs act in the central nervous system. The proposed sites of drug action described by Himwich (1951, 1958, 1961), Gangloff and Monnier (1957a and b), Monnier (1957), Monnier and Krupp (1959), and Monnier *et al.,* (1960), as well as Domino (1962), illustrate perhaps the boldest attempts to delineate the sites of action of centrally acting drugs.

Interest in drugs affecting the central nervous system arises primarily from application to human therapy, or because of abuse by psychically or physically dependent individuals. Of necessity, most biochemical, psychological, and pharmacological studies of these phenomen must be performed using various animals. This immediately presents us with the problem of inter- and intraspecies variation, especially in regard to drugs affecting the central nervous system. Variations in brain complexity further increase the problem. There are several additional sources of difficulty. (1) There is a tendency on the part of most investigators to use their own pet techniques involving one or other animal species. Investigators seldom use a wide variety of animal species to determine if the effects observed apply to a majority or to simply one of the available experimental animals. Much too often, attempts are made to transfer data from one animal species to man without knowing whether the effects observed are species specific. (2) The problems related to dosage and duration of therapy must be considered. Frequently it is not clear whether the effects described are related to physiologic, pharmacologic, or toxicologic amounts of medication. (3) Research methods in neurophysiology frequently involve either drastic surgical procedures which may produce brain injury or chronic indwelling brain electrodes which may produce glial scars. (4) Further complications arise because of the use of experimental techniques which are difficult to quantify. Neurophysiological techniques frequently are too gross (such as recording spontaneous EEG activity) or too fine (microelectrode recordings of single units) to provide more than the crudest hypotheses of the neurological actions of psychoactive drugs. Inasmuch as behaviour of an organism represents a complex interaction of many functional neuronal systems it follows that drug modifications of behaviour probably are the result of chemical interaction with neurons and/or glia at many levels of the nervous system. Most drugs affecting behaviour produce a spectrum of phenomena which result from neuronal involvement at many levels of the nervous system. This is particularly true of alcohol, barbiturates, antianxiety and antipsychotic drugs.

Neuroanatomical and neurophysiological data demonstrate that each area of the central nervous system itself is a microcosmos of highly differentiated cells with entirely different inputs and outputs. Histochemical studies of even 1,000 to 10,000 densely packed neurons in 1 cubic mm of cerebral cortex emphasize the marked chemical differences present among adjacent cells. Neuropharmacological studies lend support to the differential sensitivity of various neurons to specific chemicals. Consider for a moment the marked variations in chemical sensitivity of neurons of the brainstem reticular formation. This exceedingly heterogeneous structure contains neurons which make up the respiratory area, the vasomotor area, the chemoreceptor trigger zone, descending bulbar facilitatory and bulbar inhibitory areas, as well as complex ascending asynchronizing and synchronizing systems. Within a 1 cm thick slice of lower brainstem reticular core are millions of neurons with diverse physiological functions.

There is considerable evidence that these neurons differ in chemical sensitivity as well. Neurons of the respiratory area are especially sensitive to stimulation by low concentrations of CO_2 and to depression by narcotic analgesics such as morphine. The chemoreceptor trigger zone is especially sensitive to stimulation by apomorphine, which induces vomiting. Neurons of the brainstem facilitatory areas appear to be depressed more by chlorpromazine than do neurons of the inhibitory areas. The reverse is true with phencyclidine. Vasomotor neurons are much more sensitive to chlorpromazine than are respiratory neurons. In low doses barbiturates depress reticular neurons of the ascending arousal system more than reticular neurons involved in respiration and vasomotor functions. Valdman (1962) in particular has shown that the various morphological elements of the reticular formation differ greatly in their sensitivity to drugs such as chlorpromazine and analgesics. As a result of such data it is obviously insufficient to state that a drug acts at a spinal, medullary, mesencephalic, thalamic, limbic, or cortical level. The specific functional system affected by the drug must also be elaborated. The task of our biochemical colleagues is difficult indeed, for in order to provide evidence for a cellular action of a drug one must show that the chemical selectively affects one reticular neuron and not another involved in a different physiological mechanism. Future biochemical pharmacologists may have to selectively isolate neurons of a discrete functional type in order to prove the selective action of a drug. The approach of present-day biochemical pharmacologists who use large aggregates of neuronal tissue, usually homogenized, to determine the effects of relatively high drug concentrations for *in vivo* conditions seems quite erroneous.

Although there is evidence for neuronal selectivity of drug action, there is also abundant evidence that some drugs are non-selective in their effects. Indeed most sophisticated neurophysiologists have long convinced themselves that drugs are ‘dirty’ tools. Perhaps it is not too surprising to suggest that as of to-day we are still quite ignorant of the precise sites and mechanisms of action of almost all drugs affecting the central nervous system. The past decade in particular has seen a remarkable increase in the amount of basic research on the neural mechanisms of drug action. So much has been published on the neuropharmacological aspects of the drugs under consideration at this symposium that it is difficult to provide a comprehensive survey of the literature. This review covers only those papers which have come to my attention. No thorough literature survey has been attempted. Apologies are offered to those whose publications have escaped my cursory survey.

As Grundfest (1964) emphasized, neuropharmacology to a large extent is devoid of any comprehensive theories of drug action. Most researchers appear to be engaged in data collecting and reporting; they usually shy away from theories of drug action. Perhaps this is because many researchers feel intuitively that the present state of the science prohibits any but the most naive theories. Nevertheless, theories are essential for they suggest new and unique experiments. The time has come to extend electrophysiological theories to involve drugs such as alcohol, the barbiturates and other psychosedatives. Perhaps this conference will facilitate such a development. Because of a lack of a comprehensive theory, the approach taken in this review will be along more conventional lines. Each class of agents will be discussed and subsequently an attempt will be made to draw conclusions as to where these agents act in the central nervous system.

ETHYL ALCOHOL

Ethyl alcohol appears to depress the cerebral cortex. The spontaneous EEG is altered depending upon the amount administered. Low doses in man decrease the *alpha* voltage and increase the voltage of *beta* waves. Slow waves appear with depression of consciousness, although even with coma some of the 10 to 20 cps activity remains (Gibbs *et al.,* 1937). The EEG effects of alcohol are somewhat similar to those of diethyl ether. Davis *et al.* (1941) have correlated blood alcohol levels with EEG effects in man. Levels of 125 mg/100 ml of blood were associated with gross evidence of alcohol intoxication including ataxia, vomiting, and occasional stupor. During alcohol-stupor-induced sleep the EEG resembled that of natural sleep. In the drunken state brief episodes of 4 to 8 cps were seen, occurring with high as well as low blood levels of alcohol; the slow waves were most obvious in the frontal areas of the brain. Davis *et al.* further showed that the EEG frequency spectrum was displaced toward the slow side. Additional findings by Engel and Rosenbaum (1945) are consistent with this concept. Engel *et al.* (1945) also pointed out that a slight decrease in mean EEG frequencies is correlated with an early stage of alcohol intoxication, similar to the EEG changes due to low blood sugar (45 mg/100 ml) or low atmospheric pressure (412 mm Hg = 16,000 feet elevation). A particularly important study by Mirsky *et al.* (1941), using rabbits, suggests that the brain can adapt to increasing amounts of blood alcohol. These investigators found a correlation between gross behaviour of the animals and the decrease in frequency of the EEG waves. Repeated injections of alcohol at intervals produced a change in EEG activity followed by a return to the original pattern even though the blood alcohol level rose by 50 to 100 mg/100 ml after each injection.

Wikler (1957) reviewed much of the literature on the effects of ethyl alcohol in relation to the barbiturates and anesthetic agents such as ethyl ether. He pointed out that many recent investigations in both man and animals indicate that ethyl alcohol progressively slows the EEG diffusely, and that this is correlated with change in gross behaviour. The EEG effects of diethyl ether differ in detail from those of ethyl alcohol. Although the behavioural effects of the barbiturates and ethyl alcohol are quite similar, their EEG actions are different in that barbiturates induce much more EEG fast wave activity. Though the EEG and behavioural effects of ethyl alcohol are usually highly correlated during the early phases of acute intoxication, this is not true during the recovery phase after single doses (Mirsky *et al.,* 1941; von Hedenstroem and Schmidt, 1951), or during chronic alcohol intoxication (Isbell *et al.,* 1955; Wikler *et al.,* 1956). Wikler has also pointed out that during intoxication with bromides the EEG shows increased fast waves with low bromide levels, although with high bromide levels diffuse slow waves appear. The EEG slow waves produced by ethyl alcohol may be interpreted as a cerebral depressant effect. In certain doses in cats, Masserman (1940) has been able to demonstrate that ethyl alcohol increases the excitability of the motor cortex and the hypothalamus, although large doses act as a depressant. In contrast, diethyl ether has little depressant effect on hypothalamic reactivity, while barbiturates are markedly depressant (Masserman, 1937).

Story *et al.* (1961) determined the effects of ethyl alcohol intoxication in cats with indwelling brain electrodes and correlated these changes with blood alcohol levels. Doses below 1 ml/kg, given either intraperitoneally or intravenously, usually produced no overt neurological changes. After doses of 1 to 2 ml/kg, ataxia and light sleep were observed. The animals could easily be aroused from the alcohol-induced sleep. Doses of 3.0 to 5.0 ml/kg produced deep sleep or stupor of 2 to 5 hours' duration. Blood alcohol levels were quite similar whether the drug was given intravenously or intraperitoneally. No gross changes in the EEG from various neocortical, thalamic, limbic, or reticular areas were observed in chronic preparations with doses up to 3 ml/kg as compared with control tracings obtained during natural wakefulness and sleep. However, doses of 2 ml/kg of ethyl alcohol enhanced the initial negative deflection of the direct cortical, transcallosal, and interhippocampal responses. Cortical recruiting responses to stimulation of nucleus centrum medianum or centralis lateralis were not affected, but the cortical augmenting responses to stimulation of ventralis posterior (somatosensory relay nucleus) were markedly depressed or flattened. Comparable depression of visual cortex responses to optic tract stimulation was also obtained. Doses of ethanol larger than 2 ml/kg increased the depression of the somatosensory and visual responses and in addition increased the late surface positive component of the direct cortical response. Story *et al.* postulated that the reduction of evoked potentials was related to depression of cortical interneurons which mediate sensory inputs into cortical Golgi type I neurons.

Larrabee and Posternak (1952) have studied the effects of various alcohols and anesthetic agents on synaptic transmission through the sympathetic ganglia. Most of the anesthetics depressed synaptic transmission more readily than conduction along a nerve trunk. In contrast, ethanol and other short-chain alcohols, in concentrations lower than those which affected transmission over the synaptic pathway, depressed conduction along the nerve fibers. Evidence was obtained that the preservation of transmission across the synapse was due to a partial masking of depression by concurrent facilitation at the synapse. Low concentrations of ethyl alcohol clearly produced a transient facilitation. Longer-chain alcohols selectively depressed synaptic transmission. These investigators pointed out that the concentrations of ethyl alcohol needed to depress synaptic transmission in the ganglia were larger than those in the blood during anesthesia, indicating that some structures in the central nervous system are more sensitive to ethyl alcohol than are sympathetic ganglia. The same was true for urethane. Consistent with the notion that ethyl alcohol depresses axonal conduction is the fact that it depolarizes peripheral nerve fibers when they are exposed to its vapors (Wright, 1947). Similar effects are observed with diethyl ether vapor even though adequate levels of oxygen are present in the vapor concentrations of both diethyl ether and ethyl alcohol. The fact that some forms of transmission involving axons as well as synapses are not affected by ethyl alcohol suggests that the central effects of the drug cannot be ascribed to the depression of axonal transmission generally. The distribution of ethyl alcohol in the central nervous system has been determined. Alcohol is present more in the gray than white matter of the cat brain, although this difference appears to be related to those areas which receive a greater blood flow (Scherrer-Etienne *et al.*, 1962).

Grenell (1957) has presented evidence that ethyl alcohol in low concentrations produces stimulation of neural structures and in large concentrations depression. The local perfusion of ethyl alcohol into a pial vessel of the cerebral cortex of the cat in concentrations of 5 to 25 mM caused an increase in amplitude of the dendritic response; concentrations of 50 mM and more of alcohol produced a decrease in the response. Subsequent experiments by Grenell and O'Neill (1965), using the chelating agent EDTA and various concentrations of ethanol via the same local cortical perfusion technique, indicate that, when dendritic potentials were increased by EDTA, low concentrations of ethanol no longer caused stimulation.

Caspers (1958) has shown in rats that ethanol in doses of 0.5 to 12 g/kg caused a decrease in the spontaneous electrical activity of the reticular formation. At the same time neocortical electrical activity became more synchronized. The threshold for electrical stimulation to produce cortical patterns was also lowered significantly with small doses of ethanol and increased only with very large amounts. This research suggests that the initial action of ethanol is not in the cerebral cortex, but in some portions of the ascending reticular formation.

Ethyl alcohol has pronounced spinal cord depressant effects. In the spinal cat sciatic-nerve patellar-reflex preparation some slight selectivity in depressing polysynaptic reflexes before monosynaptic ones has been observed (Domino, 1956). However, this selectivity is negligible. By means of electrophysiological techniques in the decapitate cat, Kolmodin (1953) has demonstrated that alcohol differs from barbiturates, for example, in that both mono- and polysynaptic reflexes are equally depressed by alcohol.* A significant depressant effect was obtained with blood alcohol concentrations of 0.05 per cent. Because this can occur in decapitate cats, Kolmodin has suggested some of the effects of alcohol in man, such as disturbances in equilibrium, gait, and coordination which occur with similar blood alcohol levels, are due to an action in the spinal cord. On the basis of data in the literature, postsynaptic structures are involved in the negative (N_1) deflection of the dorsal cord potential elicited by stimulating low-threshold cutaneous fibers. Even high doses of ethyl alcohol do not affect the N_1 waves, indicating that transmission in afferent systems is more resistant than transmission through motor neurons. In this regard alcohol resembles the barbiturates.

BARBITURATES

Low doses of various barbiturates produce fast waves in the EEG of man (Brazier, 1954). Similar fast-wave activity may be observed in animals, but generally it is not as well developed. Brazier has offered evidence that fast-wave activity is recorded better from the gray matter than from underlying white matter from the frontal lobes of surgical patients. A reversal in potential of the fast-wave activity was seen in the deeper layers of the cerebral cortex indicating the superficial site of origin of these waves. Mental patients subjected to various lobotomies have a marked diminution in the amplitude of fast-wave activity induced with light thiopental anesthesia after undercut or complete isolation of a small portion of frontal cortex (Echlin *et al.,* 1952; Henry and Scoville, 1952). Although EEG fast-wave activity is best recorded from the superficial layers of the cerebral cortex, subcortical influences would appear to be important for the initiation and maintenance of such activity. Suppression bursts recorded in isolated cerebral cortex are depressed by barbiturates as is cortical afterdischarge in isolated cortex preparations. Thus, most animal evidence suggests that barbiturates depress the cerebral cortex directly.

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* Ed. note: Kolmodin found that in half of his animals the mono- and polysynaptic pathways were affected equally by ethanol, while in the other half the polysynaptic path was more sensitive.

Keller and Fulton (1931) showed in monkeys that pentobarbital and phenobarbital increase the threshold for electrical stimulation of the motor cortex. Phenobarbital was much more specific in this regard in that it caused a marked elevation of the threshold in nonanesthetic doses as compared to pentobarbital. More recently Delgado and Milhailovic (1956) have also shown that phenobarbital elevates the threshold of the motor cortex. In doses of 15 mg/kg given intraperitoneally phenobarbital caused an elevation of the threshold for minimal motor response in area 4. It also elevated the threshold in area 6 as well as in the anterior dorsal nucleus of the thalamus and in the hippocampus. Phenobarbital raised the threshold for local motor convulsions and shortened seizure duration in all these areas. The recovery time for convulsive capacity of areas 4 and 6 was also prolonged. In addition, phenobarbital elevated the threshold for electrical afterdischarge. The threshold for motor cortical afterdischarge was increased 260 per cent, that for the amygdala 160 per cent, and that of the anterior dorsal nucleus of the thalamus 351 per cent.

Gangloff and Monnier (1957a) provided evidence for the differential threshold-elevating effects of phenobarbital in the unanesthetized rabbit. In contrast to the reports of others, these investigators showed that phenobarbital given orally in doses of 30 to 60 mg/kg reduced the threshold for induced electrical afterdischarge in the sensory motor cortex. On the other hand, the threshold for electrical afterdischarge from stimulation of the lateral thalamus was markedly elevated, and the duration of afterdischarge reduced. The threshold for afterdischarge elicited in the dorsal and ventral hippocampus was also elevated but not as much. The duration of afterdischarge was not markedly altered. The finding that phenobarbital in certain doses lowered the threshold for the sensorimotor cortex is reminiscent of the study of Schutz and Caspers (1953), who showed that low doses of phenobarbital (20 to 40 mg/kg, given intramuscularly to rats) may facilitate and provoke epileptic-type electrical discharges in the cerebral cortex, particularly when the cortex is injured. Such an effect may be secondary to a release phenomenon of the cortex due to depression of ascending inhibitory systems, as proposed by various investigators.

Aston and Domino (1961) have provided additional evidence supporting the greater clinical usefulness of phenobarbital than of pentobarbital as an anticonvulsant. In monkeys with chronic indwelling brain electrodes phenobarbital and diphenylhydantoin were shown to elevate motor cortical thresholds, in doses which caused only minimal or no increase in reticular activating system thresholds for arousal. Pentobarbital, however, increased both motor cortical thresholds and reticular activating system thresholds to a similar extent. These findings are in agreement with those of Martin *et al.* (1954), who found that diphenylhydantoin in doses up to 30 mg/kg had no effect on EEG activation elicited from stimulation of the reticular formation or on the spindles of the high-pontine-transected cat preparation. However, phenobarbital in equal doses or larger (30 to 70 mg/kg given intravenously) depressed EEG activation. Doses of phenobarbital one-third as large as those used in intact animals depressed the frequency of spindles and induced more slow waves in the high-pontine brainstem-transected preparation.

The predominant EEG effect of barbiturates in acute animal preparations is the appearance of spindles and generalized slow waves in the cerebral cortex. Pentobarbital and amobarbital also cause marked EEG slowing in the caudate nucleus of cats (Hendley *et al.*, 1956). Pentobarbital and related barbiturates also produce slow waves in the isolated cerebral cortex preparation of both cat (Domino, 1957) and dog (Rech and Domino, 1960). The threshold for induced afterdischarge of the isolated cortex of dogs is elevated and its duration shortened by thiopental and phenobarbital. There is much evidence, therefore, to support the belief that barbiturates have a depressant effect directly on the cerebral cortex. This effect is, however, very dependent on dosage; under certain circumstances with small doses a facilitating effect on seizures can be shown at least electroencephalographically.

In addition to an action on the cerebral cortex, there is evidence that barbiturates either directly or indirectly affect various thalamic nuclei: phenobarbital elevates the threshold for induced afterdischarges in the anterior dorsal thalamic nucleus of monkeys (Delgado and Milhailović, 1956) and in the lateral thalamus of rabbits (Gangloff and Monnier, 1957a). As Marshall (1941) has shown, barbiturates have a depressant effect on the recovery cycles of specific relay nuclei in the thalamus. These findings have since been confirmed by King *et al.* (1957). Low intravenous doses (5 to 10 mg/kg) of thiopental and pentobarbital prolonged the latency, increased the amplitude, and broadened the shape of single evoked responses mediated through nucleus ventralis posterior. Recovery cycles were only slightly prolonged with facilitation of the amplitude of the second responses. In general, these effects were similar to those associated with EEG synchrony and therefore could be attributed to depression not of the thalamus directly but of ascending influences from the reticular formation. However, in anesthetic doses of 30 mg/kg the barbiturates markedly increased the latency, reduced the amplitude, and prolonged the recovery time of thalamic responses, suggesting a direct depressant effect on transmission through this specific relay nucleus. Similar conclusions have been made by Schwartz *et al.* (1962). Anesthetic doses of barbiturates have been shown to have a depressant action directly upon the thalamic relay nuclei for each afferent modality (King *et al.,* 1957).

There is considerable evidence that barbiturates affect responses of the diffuse thalamic projection system. In low doses, barbiturates facilitate recruiting responses following low frequency stimulation of nuclei of the diffuse thalamic projection system of cats (Domino, 1955; Jasper *et al.,* 1955; King, 1956). In anesthetic doses, recruiting responses are diminished in amplitude and there is a lack of 1 to 1 following (Domino, 1955). This is probably indicative of prolonged neuronal recovery cycles in this system as well. Cats with chronic indwelling brain electrodes given pentobarbital intravenously in 10 mg/kg doses show an elevated threshold for both EEG activation and gross behavioural arousal elicited with high frequency stimulation of various nuclei of the diffuse thalamic projection system (Killam and Killam, 1956). In contrast to the effects of chlorpromazine, pentobarbital did not produce a marked separation of gross behavioural arousal and EEG activation. These results indicate a depressant effect of pentobarbital. They are in marked contrast to the facilitating effects of pentobarbital on recruitment elicited by low frequency stimulation of the diffuse thalamic projection system. This latter action may be related to a release phenomenon caused by depression of the brainstem reticular formation (Domino, 1955).

Barbiturates are known to depress the functions of the hypothalamus. For example, temperature regulation as well as such emotional reactivity as sham rage are depressed by low doses of barbiturates. Similarly, evoked electrical potentials recorded in the hypothalamus of the cat are depressed by pentobarbital. Feldman *et al.* (1958) have shown that the hypothalamus is even more sensitive to barbiturates than is the midbrain reticular formation. Large anesthetic doses of pentobarbital caused the appearance of high voltage, long latency responses in the hypothalamus, intralaminar nuclei of the thalamus, and midbrain reticular formation following stimulation of the sciatic nerve in the cat. These slow potentials are thought to be the secondary responses of Forbes, which can be recorded at brainstem levels (Feldman and Porter, 1960). Pentobarbital in doses of 10 mg/kg slightly elevates the threshold for behavioural arousal and startle due to stimulation of the posterior hypothalamus of monkeys. Pentobarbital in 20 mg/kg doses produces a very marked elevation in electrical threshold for the same phenomenon.

The limbic system also is depressed by various barbiturates. Pentobarbital in doses of 5 to 10 mg/kg given intravenously has been shown to cause a marked elevation in threshold and a decrease in duration of afterdischarge from electrical stimulation of the amygdala or hippocampus of cats (Killam *et al.*, 1957). Similar findings have been reported by Takagi and Ban (1960), who used hexobarbital in doses of 10 to 20 mg/kg intravenously in high spinal cat preparations. Repetitive electrical stimulation of the precommissural fornix results in hippocampal electrical seizures. Following hexobarbital such electrical seizures were markedly reduced in duration, with an elevation in threshold. On the other hand, single shock stimulation of the fornix elicits a potential in the hippocampus which is not affected by 10 mg/kg of hexobarbital. However, post-tetanic potentiation of this response is blocked by such doses of hexobarbital. Takagi and Ban also demonstrated that hexobarbital produced similar effects on post-tetanic potentiation elicited by stimulation of the amygdala on potentials recorded in the hippocampus, and post-tetanic potentiation elicited by stimulation of the hippocampus on potentials recorded in the lateral hypothalamus. Aston and Domino (1961) have shown that both pentobarbital and phenobarbital elevate the threshold for electrical afterdischarge in the hippocampus of monkeys. Delgado and Milhailović (1956) have shown that these structures as well as the amygdala have an elevation of the threshold for electrical afterdischarge following phenobarbital. As noted above, the elevations in threshold were not nearly as marked as those in the thalamus.

A great deal of evidence indicates that small amounts of barbiturates and other anesthetics depress reticular neurons involved in the brainstem activating system. Brazier (1954), French (1960), and Killam (1962) reviewed the literature on the depressant effects of barbiturates and other anesthetic agents on this system. Barbiturates in low doses depress EEG activation elicited by stimulation of the brainstem reticular formation in a variety of animal species. King *et al.* (1957) have shown that the relative recovery period of responses to peripheral nerve stimulation recorded from the midbrain reticular formation of cats is also depressed by small doses of pentobarbital. In cats with chronic brain electrodes, pentobarbital raises the threshold for behavioural arousal more than the threshold for EEG desynchronization (Killam *et al.*, 1957).

Barbiturates have also been shown to have depressant effects upon the spinal cord. Small doses of pentobarbital and diallylbarbituric acid depress polysynaptic pathways more than monosynaptic pathways. This has been shown in various animal species (see review by Domino, 1956). Phenobarbital in intravenous doses of 2.5 to 10 mg/kg has been shown to depress the flexor reflex of high spinal cats (Pfeiffer *et al.*, 1957). Anesthetic doses of pentobarbital depress both mono- and polysynaptic reflexes and stabilize the membrane potential of spinal neurons (Eccles, 1946). Using decapitated cats, Wilson and Talbot (1960) studied the effects of intravenous pentobarbital on recurrent facilitation and inhibition of monosynaptic potentials elicited by stimulating dorsal roots and recording potentials from cut peripheral nerves. Recurrent facilitation or inhibition was initiated by antidromic impulses entering the cord via the intact ventral roots. Pentobarbital in intravenous doses of 5 to 10 mg/kg abolished recurrent facilitation but did not decrease recurrent inhibition. These effects were accompanied by a marked depression of the monosynaptic reflex in contrast to similar studies using meprobamate (see discussion of the action of meprobamate on the spinal cord). More recently Eccles (1963, 1964) and his associates have shown that barbiturates such as pentobarbital, as well as chloralose, increase and prolong both presynaptic inhibition and associated presynaptic depolarization of afferent terminals in the spinal cord. After massive doses of these agents presynaptic inhibition and depolarization are even more markedly prolonged. Eccles has suggested several explanations for this: the prolongation of presynaptic inhibition by barbiturates could be caused by inactivation of an enzyme responsible for destroying a chemical transmitter substance, or barbiturate-induced prolongation of presynaptic depolarization could be due to an increased effectiveness of the diffusional barrier which normally restricts diffusion of the transmitter substance. Schmidt (1963) has studied the effects of various drugs on primary afferent depolarization in the toad spinal cord. Anesthetics such as ether, paraldehyde, urethane, and chloral hydrate are quite different in their action from the barbiturates and chloralose, inasmuch as the former drugs produce only a shortening and diminution of primary afferent depolarization. The fact that of all these anesthetics only chloralose and barbiturates prolong presynaptic inhibition, favours the possibility that the latter drugs interfere with enzyme inactivation of the transmitter substance involved in presynaptic inhibition. Recently many investigators have demonstrated that presynaptic inhibition seems more effective than postsynaptic inhibition at most of the excitatory synapses of primary afferent fibers. This is in accord with the general concept proposed by Eccles (1963) that presynaptic inhibition functions as a negative feedback on the inflow of sensory information into the central nervous system. In contrast to the effectiveness of barbiturates in prolonging presynaptic inhibition, postsynaptic inhibition is not affected by these compounds but is blocked by strychnine and tetanus toxin. Furthermore, presynaptic inhibition is not affected by strychnine, but is blocked by picrotoxin. Thus the pharmacological evidence clearly distinguishes these forms of inhibition, which is in agreement with other physiological evidence.

MEPROBAMATE

Meprobamate induces fast waves, 20 to 30 cps, in the cortical EEG of man, similar to those seen after barbiturates. Oral doses of 1600 to 2000 mg induce such activity, particularly in the parietal areas (Pfeiffer *et al.,* 1957). This EEG fast wave activity has been observed by many investigators (Bokonjic and Trojaborg, 1960; Henry and Obrist, 1958; Henry *et al.,* 1957; Miletto *et al.,* 1956; Tucker and Wilensky, 1957). Bokonjic and Trojaborg (1960) have studied the effects of a wide range of dosage of meprobamate (50 to 400 mg/kg orally) on the EEG of patients during treatment, intoxication, and after abrupt withdrawal. They have pointed out that in many respects meprobamate and the barbiturates have similar effects on the EEG. Both drugs induce fast wave activity which is more pronounced during drowsiness and disappears during sleep. EEG evidence of tolerance to both drugs was demonstrated. After abrupt withdrawal of both agents paroxysmal EEG changes are noted during photic stimulation. The cortical distribution of the fast wave activity induced by meprobamate seems to differ somewhat, however, from that induced by barbiturates. After meprobamate this activity tends to be more pronounced in the parietal areas, while after barbiturates it is more pronounced frontally, frontotemporally, or parietally. After withdrawal from meprobamate, patients show paroxysmal EEG changes which may persist in rare circumstances for as long as two weeks. In contrast, after withdrawal of barbiturates the EEG changes may last as long as two months. The principal EEG differences between meprobamate and barbiturates occur after injection of toxic doses. In meprobamate poisoning the fast waves persist even during the period of unconsciousness. In contrast EEG fast wave activity persists only with small doses of barbiturates. Frequencies of less than 6 cps rarely are seen after meprobamate poisoning. The EEG usually is normal within 48 hours after even such large doses as 400 mg/kg orally. After large toxic doses of meprobamate the fast wave activity is replaced by 2 to 6 cps activity of high amplitude. It may take weeks for the EEG to become normal, although some of this could be explained as being secondary to brain damage due to hypoxia from respiratory depression.

Meprobamate also has been shown to cause a slight increase in the fast components of the EEG in animals. In intact cats immobilized with gallamine, meprobamate in the order of 400 mg/kg intravenously is necessary to produce this phenomenon. On the other hand, doses of 10 to 30 mg/kg intravenously do not consistently alter spontaneous EEG activity of either the cortex or subcortex (Gangloff, 1959). These findings indicate that the drug produces effects somewhat similar to those of the barbiturates, but meprobamate is much less potent. Although meprobamate has not been studied in isolated cortex preparations, one would expect, in view of its similarities to certain barbiturates, that it would have direct cortical depressant effects.

In intravenous doses of 20 mg/kg to intact curarized cats, meprobamate produces selective synchronization of the spontaneous EEG activity in various specific and diffusely projecting thalamic nuclei, including the lateral geniculate, nucleus ventralis lateralis, nucleus ventralis posterior medialis, and nucleus centre median (Hendley *et al.*, 1956). These investigators observed that thalamic EEG slowing was one of the earliest manifestations of meprobamate. In cats and humans, similar slow wave activity following meprobamate has been seen in the caudate nucleus, pallidum, and amygdala (Baird *et al.*, 1957). EEG slow waves have also been seen in nucleus lateralis posterior of the thalamus of monkeys following 20 mg/kg of meprobamate given intravenously (Berger *et al.*, 1957). These changes have been claimed to be characteristic of meprobamate and different from those of other central nervous system depressants. Frequent waxing and waning and occasional spindling were observed. After large doses of meprobamate

(120 mg/kg), generalized slow waves of increased amplitude were observed. Even after these large doses, the thalamus showed greater changes. In contrast to these effects of meprobamate, 5 mg/kg of pentobarbital produced generalized slowing and spindling, more apparent in the cerebral cortex and caudate nucleus than in the thalamus or hypothalamus. Amobarbital in similar dosage in two cats was reported to cause more high voltage activity in the thalamus than pentobarbital. Mephenesin in doses of 40 mg/kg had no significant effect. Chlorpromazine in doses of 10 mg/kg intravenously and reserpine in doses of 2.5 mg/kg yielded inconsistent effects, although thalamic slowing sometimes was seen. In intact cats immobilized with gallamine, Gangloff (1959) observed that meprobamate in intravenous doses as low as 10 mg/kg elevated the threshold of EEG recruitment following stimulation of nucleus centralis lateralis of the thalamus. However, after lesions of the midbrain reticular formation, meprobamate even in doses of 50 mg/kg had no effect on the threshold for recruitment. Takaori and Ohata (unpublished observations) have found that meprobamate in doses of 20 to 50 mg/kg given intravenously produced no change in recruiting responses (recorded from the cerebral cortex) elicited by low frequency stimulation of nucleus centre median in high spinal cats. Doses of 80 mg/kg increased EEG slow waves and background activity, obscuring any effect on recruitment.

Meprobamate gives evidence of a depressant action on the hypothalamus. Bovet *et al.* (1957) studied the effects of meprobamate in intravenous doses from 10 to 100 mg/kg on EEG arousal and the alarm reaction of rabbits in which the hypothalamus was stimulated. They showed that meprobamate inhibited the alarm reaction and partially depressed behavioural arousal from hypothalamic stimulation.

Meprobamate also depresses the activity of the limbic system. In intravenous doses of 20 mg/kg it causes a decrease in electrical afterdischarge in both the amygdala and hippocampus of acute cat preparations (Kletzkin and Berger, 1959). The fact that these effects occur after relatively low doses suggests a somewhat more selective depressant action on the limbic structures of the brain. Takagi and Ban (1960) have been unable to show that 20 mg/kg of meprobamate given intraperitoneally has any suppressant effect on hippocampal afterdischarge produced by repetitive stimulation of the precommissural fornix of high spinal cats. On the other hand, after 40 mg/kg of meprobamate the duration of seizures was slightly shortened without any change in threshold. These two reports are somewhat consistent in that larger, intraperitoneal doses of meprobamate would be expected to produce the same effects as smaller intravenous doses. Following 20 mg/kg of meprobamate given intravenously, Schallek and Kuehn (1960) have reported a shortening of afterdischarge caused by electrical stimulation of the septum in intact cats immobilized with decamethonium. All of these observations clearly indicate that meprobamate has depressant effects on the limbic system.

It is therefore of interest that doses of 20 mg/kg of meprobamate which depress limbic system afterdischarge have negligible effects on the threshold duration of EEG arousal from stimulation of the reticular formation of the brainstem. Kletzkin and Berger (1959) have pointed out that 20 mg/kg of meprobamate has no significant effect on cerebral cortical and hippocampal EEG activation elicited by stimulation of the reticular formation in post-ether anesthetized cats. They have pointed out that this is quite different from the effects of various barbiturates which depress both limbic system afterdischarge and EEG arousal. The relative doses administered were particularly important. Further work along these lines is indicated. In intact cats immobilized with gallamine, Gangloff (1959) observed that meprobamate in intravenous doses up to 80 mg/kg did not usually alter the threshold of EEG activation from stimulation of nucleus centralis lateralis of the thalamus, the midbrain reticular formation, and sciatic nerve. Only in doses of 100 to 200 mg/kg, which are reported to induce sleep, did the threshold for EEG activation increase slightly. Animals with midbrain reticular lesions given low doses of meprobamate (10 to 20 mg/kg intravenously) inconsistently showed increases in the threshold for EEG activation following stimulation of the sciatic nerve, rostral reticular formation, and nucleus centralis lateralis of the thalamus. Takaori and Ohata (unpublished studies, 1961), observed that 20 mg/kg of meprobamate given intravenously had no effect on EEG arousal following stimulation of the midbrain reticular formation in high spinal cats. Larger doses (50 to 80 mg/kg) shortened the duration of EEG activation. These studies indicate that meprobamate has less depressant effects on the brainstem reticular formation than do the barbiturates.

Perhaps the most important point to note is that the slope of meprobamate's dose-effect curve is less steep than that of the barbiturates. In cats paralyzed with succinylcholine, Kletzkin and Swan (1959) compared the effects of meprobamate with those of pentobarbital on cortical, medial geniculate, nucleus centrum medianum, nucleus medialis dorsalis, midbrain reticular formation and amygdala potentials evoked by auditory click stimuli. Doses of 40 mg/kg of meprobamate given intravenously had no effect on the responses from the specific ascending system (recorded from the medial geniculate and auditory cortex), but the amplitude of the responses from the nonspecific afferent system (recorded from the reticular formation, centro-median nucleus and amygdala) was slightly enhanced by meprobamate. Doses of 80 mg/kg caused little change, except for an enhancement of about 30% in the evoked responses from the reticular formation. Unfortunately, Kletzkin and Swan used relatively large anesthetic doses of pentobarbital (30 to 40 mg/kg). As might be expected, they were able to abolish or depress responses in nucleus centrum medianum, nucleus dorsalis medialis, and the reticular formation; the amplitude of the evoked responses in the specific relay system was also depressed by about one-third. Unfortunately, large anesthetic doses of meprobamate were not studied, so a valid comparison to the barbiturates is not possible.

Meprobamate also has depressant effects upon the spinal cord. In chloralose- or diallylbarbiturate-anesthetized cats, 40 mg/kg of meprobamate given intravenously depressed both patellar and flexor reflexes (Berger, 1954). Polysynaptic reflexes such as the flexor, crossed extensor, and linguomandibular reflexes were more depressed than monosynaptic reflexes such as the patellar. The onset of action of meprobamate was much slower than that of mephenesin. Subsequently, Hendley *et al.* (1954) showed that in similar preparations meprobamate in intravenous doses of 50 to 80 mg/kg usually had no effect on the amplitude of the patellar reflex, but it abolished the flexor reflex.

Abdulian *et al.* (1960) compared the effects of meprobamate with those of mephenesin, pentobarbital, and SKF 1045 on inhibition and facilitation of the patellar reflex evoked by stimulation of the ipsilateral and/or contralateral sciatic nerve in high spinal cats. As a guide for dose levels to be used in the cat experiments, relative potency for producing ataxia, loss of the righting reflex, and death was determined in mice. The effects of mephenesin and SKF 1045 differed from those of meprobamate and pentobarbital. In doses producing ataxia in mice, mephenesin and SKF 1045 produced only a slight decrease in the amplitude of the patellar reflex in cats. On the other hand, in subataxic doses in mice, meprobamate and pentobarbital produced a depression of the patellar reflex comparable to that of mephenesin and SKF 1045. Inhibition of the patellar reflex was depressed by all drugs studied. The depression of inhibition occurred with subataxic doses which did not markedly depress the patellar reflex, but did depress its facilitation. In spinal cats, Pfeiffer *et al.* (1957) have shown that meprobamate in intravenous doses of 40 mg/kg abolished the flexor and depressed the patellar reflex. Comparable effects were produced by 2.5 mg/kg of phenobarbital or 20 mg/kg of mephenesin. DeSalva and Ercoli (1959) compared the effects of meprobamate with those of styramate, phenaglycodal, and chlorpromazine on the augmentation of the patellar reflex by contralateral sciatic nerve stimulation (polysynaptic) and the patellar reflex itself (monosynaptic) in decerebrate and spinal cats. A 27 to 35 mg/kg intravenous dose of meprobamate blocked the effect of sciatic nerve stimulation to a similar extent in decerebrate and spinal animals without affecting the patellar reflex. In contrast, styramate, phenaglycodal, and chlorpromazine were much more effective in depressing the effects of sciatic nerve stimulation in decerebrate animals than in spinal ones. DeSalva and Oester (1960) extended these findings by comparing the actions of meprobamate and other central nervous system depressants on the effects of contralateral and ipsilateral stimulation of the sciatic nerve on the patellar reflex in high spinal cats. They concluded that polysynaptic reflexes are more depressed than monosynaptic reflexes, but monosynaptic reflexes are also depressed by appropriate doses of a variety of centrally acting drugs.

Wilson (1958) has shown that the monosynaptic flexor and extensor reflexes of the decapitate cat, in which selected peripheral muscle nerves are stimulated and the potentials recorded in the ventral root, are depressed after cumulative intravenous doses of 100 mg/kg of meprobamate. Initial doses of 30 to 40 mg/kg had unpredictable actions. Inhibitory pathways, both direct and disynaptic, were highly resistant to meprobamate. Furthermore, the drug did not affect the input-output relations of monosynaptic reflexes. Wilson suggested that meprobamate acts as a general depressant of excitatory synaptic transmission. Wilson and Talbot (1960) studied the effects on recurrent facilitation and inhibition of monosynaptic potentials, of meprobamate administered intravenously in cumulative doses of 40 mg/kg every 40 minutes to decapitate cats. Recurrent facilitation or inhibition was initiated by antidromic impulses entering the cord via the intact ventral roots. Total doses of 210 to 400 mg/kg of meprobamate were necessary to abolish recurrent facilitation. Such large doses did not decrease recurrent inhibition and in fact prolonged the duration of the inhibition. As described earlier, pentobarbital had somewhat similar actions to meprobamate on recurrent conditioning. However, these effects were accompanied by a more marked depression of the monosynaptic reflex, in contrast to the doses of meprobamate, which had the same action on conditioning.

BENZODIAZEPINES

Chlordiazepoxide and diazepam are representatives of the benzodiazepine class of drugs which have psychosedative properties. The drugs appear to have relatively shallow dose-response curves for central nervous system depression. These substances have been claimed to have taming effects in monkeys in doses below those which produce ataxia, in contrast to the effects of compounds such as chlorpromazine, meprobamate, and phenobarbital. The benzodiazepines have many properties in common with meprobamate and phenobarbital; on the other hand, they are claimed to be much less hypnotic than other compounds. Relatively few neuropharmacological studies on the benzodiazepines have been published except for those by the investigators involved in the development of these compounds. Schallek and Kuehn (1960) have shown that 10 mg/kg doses of chlordiazepoxide given intravenously to intact cats immobilized with decamethonium depress the duration of electrical afterdischarge from stimulation of the septum, amygdala, and hippocampus. The compound is more potent than meprobamate in depressing the irritability of rats with septal lesions. Himwich *et al.* (1962) have compared the effects of chlordiazepoxide, diazepam, meprobamate, and pentobarbital on the amygdala and hippocampus of rabbits, using as endpoints spontaneous EEG and elicited afterdischarge. They concluded that both meprobamate and pentobarbital depressed these structures, while chlordiazepoxide and diazepam depressed the amygdala but appeared to stimulate the hippocampus.

Large doses of chlordiazepoxide block EEG activation from stimulation of the brainstem reticular formation (Randall, 1960); the drug would appear to have a more depressant effect on the limbic system than it does on the brainstem activating system or cerebral cortex. The benzodiazepines also have actions in the spinal cord. Depression of the flexor reflex in chloralose-anesthetized cats is observed after 3 mg/kg of chlordiazepoxide given intravenously; depression of EEG activation occurs in doses considerably above those which blocked the flexor reflex. In contrast, the dose of meprobamate required for blocking spinal reflexes is 50 mg/kg given intravenously, with depression of EEG arousal requiring 40 mg/kg, while the dose of phenobarbital required to block EEG arousal is approximately 1 mg/kg, and that causing flexor reflex blockade is 15 mg/kg. These findings suggest that chlordiazepoxide produces depression of the flexor reflex more selectively.

CHLORPROMAZINE

Chlorpromazine does not appear to have any significant cortical effects. Preston (1956) found that even massive doses of chlorpromazine (50 mg/kg intravenously) had no significant depressant effects upon the isolated cortex of the cat. In doses up to 8 mg/kg in high spinal-transected cat preparations, chlorpromazine had no significant effect on transcallosal potentials recorded from the lateral gyrus (Takaori, 1958). Although chlorpromazine itself does not block the transcallosal response of the lateral gyrus, it and related phenothiazines prevent catecholamine-, serotonin-, mescaline-, and LSD-25-induced depression of this potential (Marrazzi, 1957). With many phenothiazines this antagonism parallels clinical potency (Marrazzi *et al.,* 1958). Delgado and Milhailović (1956) were unable to find a significant change in the threshold of stimulation for minimal motor movements or electrical afterdischarge of areas 4 and 6 after 1 mg/kg of chlorpromazine in monkeys with chronic indwelling brain electrodes. With 5 to 10 mg/kg intravenous doses of chlorpromazine to unanesthetized rabbits, Gangloff and Monnier (1957b) showed that the threshold was elevated and also electrical afterdischarge was prolonged on stimulation of the sensorimotor cortex. In unanesthetized high spinal cats, chlorpromazine in intravenous doses of 3 mg/kg depressed the second component of the local cortical response to stimulation of the lateral gyrus (Takaori, 1958). In pentobarbital-anesthetized cat preparations the cortical evoked response to stimulation of the splanchnic nerve was depressed by chlorpromazine in intravenous doses of 5 mg/kg. These latter effects are similar to those produced by morphine. Evoked potentials recorded in the cortex following stimulation of the sciatic nerve are enhanced throughout the cortex following chlorpromazine in intravenous doses of 5 mg/kg (Unna and Martin, 1957).

Chlorpromazine has no significant effects on the specific thalamic relay nuclei. In contrast to the effects of pentobarbital, the Killams (1957, 1959) were unable to show any significant effect of chlorpromazine in doses up to 5 mg/kg on recovery cycles through nucleus ventralis posterior of cats. Preston (1956) made similar observations using doses up to 50 mg/kg. Takaori (1958) was able to show that chlorpromazine in intravenous doses of 3 mg/kg depressed the augmenting response recorded from the suprasylvian gyrus to repetitive stimulation of the medial lemniscus of high spinal unanesthetized cat preparations. The threshold for electrical afterdischarge induced by stimulation of the lateral thalamus in unanesthetized rabbits is slightly decreased by 5 to 10 mg/kg of chlorpromazine, and the duration of afterdischarge is prolonged (Gangloff and Monnier, 1957b). In monkeys with chronic indwelling brain electrodes, chlorpromazine in doses of 1 mg/kg lowered the threshold to nearly one-half of its original value for eliciting afterdischarge due to stimulation of nucleus anterior dorsalis of the thalamus (Delgado and Milhailović, 1956).

Chlorpromazine has a slight diphasic effect upon the diffuse thalamic projection system. Das *et al.* (1955) reported that chlorpromazine decreased the incidence of spindles in the *cerveau isolé* cat preparation. Killam and Killam (1956) reported that chlorpromazine in doses of 1 mg/kg in unanesthetized cat preparations slightly enhanced cortical recruitment, but doses of 2 to 8 mg/kg depressed it slightly. In contrast to low-frequency stimulation of the diffuse thalamic projection system which causes EEG recruiting and sleep, high-frequency stimulation causes EEG and behavioural arousal. Chlorpromazine in doses of 5 mg/kg elevates the threshold for behavioural arousal in cats following high-frequency stimulation of this system. It appears to elevate the threshold for gross behaviour more than the threshold for EEG activation. Gangloff and Monnier (1957b) reported that in the unanesthetized rabbit preparation chlorpromazine in 5 mg/kg intravenous doses produced an increase in the amplitude of recruiting responses to low-frequency stimulation of nuclei of the diffuse thalamic projection system.

Chlorpromazine also depresses the hypothalamus. Dasgupta *et al.* (1954) reported that chlorpromazine in intravenous doses of 100 to 250 μg/kg suppressed sham rage in diencephalic cats. In fact, the animals showed increased sensitivity to the drug. Thiopental in intravenous doses of 2.5 mg/kg produced somewhat similar effects. Dasgupta and Werner (1954) have shown that chlorpromazine in intravenous doses of 0.5 to 1 mg/kg produced irregular depression of the blood pressure responses to stimulation of the hypothalamus and medullary vasomotor areas of intact chloralose-anesthetized cats. The role of peripheral adrenergic or ganglionic blockade was not adequately evaluated. However, similar pressor responses were uniformly abolished in decorticate cats after much smaller doses of chlorpromazine (50 to 100 μg/kg intravenously), suggesting increased sensitivity to the drug following decortication. Longo (1956) reported that chlorpromazine depressed EEG activation as well as the fright and flight reaction caused by stimulation of the hypothalamus of rabbits with chronic indwelling brain electrodes. There is also evidence that chlorpromazine inhibits hypothalamo-neurohypophysial secretion (Shibusawa and

Fukuda, 1955). Barraclough (1956) reported that chlorpromazine blocks the release of pituitary gonadotropin in rats.

Chlorpromazine appears to have mixed stimulant and depressant effects on the limbic system. Preston (1956) and Takaori (1958) have shown that very large doses of chlorpromazine (40 mg/kg intravenously) increased spontaneous seizure discharge in the amygdala. In contrast to the effect of such massive doses, smaller doses of chlorpromazine (5 mg/kg) shortened motor convulsant patterns on stimulation of either the amygdala or hippocampus when the seizure involved cortical as well as rhinencephalic structures (Killam *et al.,* 1957). Although chlorpromazine neither altered the threshold nor changed the duration or pattern of the rhinencephalic electrical discharge, it reduced the clonic component by at least a third. A larger dose (8 mg/kg intravenously) appeared to prolong both phases of the seizure. Chlorpromazine, in doses which markedly depressed unanesthetized cats, increased the threshold for limbic system afterdischarge (Killam, 1956). Takagi and Ban (1960) have also shown that hippocampal afterdischarge to repetitive stimulation of the precommissural fornix of high spinal cats is depressed by low doses of chlorpromazine (1 to 3 mg/kg intravenously). After this dose the afterdischarge was completely suppressed; it recovered about 1 hour later. Chlorpromazine in intravenous doses of 1 to 5 mg/kg had no effect on the hippocampal potential to single shock stimuli to the fornix or its post-tetanic potentiation. It also had no effect on the evoked potential in the hippocampus to single shock stimuli to the basolateral nuclei of the amygdala. However, chlorpromazine in doses of 1 mg/kg depressed the development of post-tetanic potentiation. In intravenous doses of 1 to 3 mg/kg it had no significant effects on the evoked potential in the hypothalamus to single shock stimuli of the hippocampus or its post-tetanic potentiation.

Killam and Killam (1956) have shown that chlorpromazine in doses of 2 to 8 mg/kg depressed EEG activation within the limbic system, as indicated by slow, high-voltage waves. The slow waves were often replaced by fast-wave activity. Delgado and Milhailović (1956) observed in monkeys with chronic indwelling brain electrodes that chlorpromazine in doses of 1 mg/kg intramuscularly reduced the threshold for evoked afterdischarge from stimulation of the amygdala. This effect was less marked than the lowering of threshold for evoked afterdischarge from nucleus anterior dorsalis of the thalamus. Adey and Dunlop (1960) studied the responsiveness of single unit discharge in the caudate nucleus and globus pallidus of the cat to sciatic nerve or amygdala stimulation before and after chlorpromazine in intravenous doses of 2 mg/kg. Chlorpromazine decreased basal firing in most pallidal units. This effect is similar to its actions in reducing the basal firing rate of reticular units (Bradley, 1957). Adey and Dunlop (1960) noted that whereas sciatic nerve stimulation either had no effect or produced a slight decrease in the firing rate of units during the control period, the same units responded with a marked increase in firing after chlorpromazine. A similar 'release' of pallidal-unit excitability was noted in the amygdala and especially to paired lateral amygdaloid-sciatic stimuli. Adey and Dunlop suggested that chlorpromazine effects involve an enhancement of the responsiveness of neural units in the basal ganglia and the reticular formation. This does not appear to be an increase in central excitatory state directly in view of the fact that basal firing rates were depressed, but rather as though some inhibitory mechanism which is operative after stimulation were depressed. Gangloff and Monnier (1957b) demonstrated that chlorpromazine in doses of 5 to 10 mg/kg given intravenously to rabbits increases the threshold and duration of afterdischarge to hippocampal stimulation. There is a blockade of EEG slow waves in the hippocampus from sciatic nerve stimulation following small doses of chlorpromazine given intravenously (Killam, 1956; Killam *et al.*, 1957).

The actions of chlorpromazine on the brainstem reticular formation appear to be extremely complex; these have been reviewed by Killam (1962). Hiebel *et al.* (1954) have shown that small doses of chlorpromazine depress EEG activation in cats with prebulbar transections. Midpontine cat preparations which showed EEG activation to epinephrine no longer showed epinephrine-induced activation after chlorpromazine. Unna and Martin (1957) have also shown that chlorpromazine blocks epinephrine-induced EEG activation. Following low to moderate doses of chlorpromazine the threshold to sensory activation is elevated (Bradley, 1958).* The threshold of EEG activation resulting from direct stimulation of the brainstem reticular formation is not altered, but the duration of arousal and activation may be decreased. Large doses of chlorpromazine may elevate slightly the threshold for EEG activation. Such effects are clearly much less marked than those produced by barbiturates.

Rinaldi and Himwich (1955) have shown that although doses of chlorpromazine under 6 mg/kg depressed EEG activation in the rabbit, doses over 15 mg/kg produced EEG activation. Himwich *et al.* (1956) have shown in a series of phenothiazines that the ability to depress EEG activation in the rabbit correlates with the clinical efficacy of these compounds in man. More recently, Himwich and his associates have shown that chlorpromazine depresses EEG activation produced by eserine. Therefore, the drug acts not only to block adrenergic mechanisms in the activating system but also to block the cholinergic activation system. Yamamoto (1959) has shown that small doses of chlorpromazine facilitate activated sleep in the cat with chronic indwelling brain electrodes.

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*Ed. note: Recently Key (1965) has reported that chlorpromazine does not depress reticular neurons directly, but blocks afferent collateral input to them.

DeMaar *et al.* (1956) and Unna and Martin (1957) have used paired shocks to the sciatic nerve in order to determine neuronal recovery cycles in the reticular formation. Two types of potentials, one of short and the other of long latency, were recorded in the reticular formation in the vicinity of the red nucleus. Chlorpromazine increased the amplitude of both the short and long latency evoked potentials; it markedly depressed the recovery cycle of the long latency response and had much less of an effect on the short latency potentials. The second potential of each pair was depressed. Somewhat similar findings have been made by the Killams (1958, etc.). Chlorpromazine enhanced the amplitude of the first potential of each pair; it likewise prolonged the reticular neuronal recovery cycles. In contrast, the amplitude of both the first and second responses of each pair was reduced by low doses of pentobarbital (5 mg/kg intravenously). The second potential was reduced more than the first, again consistent with prolongation of neuronal recovery cycles.

The Killams have shown that chlorpromazine in low doses enhances reticular inhibition of sensory input. Reticular stimulation is known to decrease the auditory-evoked response in the cochlear nucleus and medial geniculate. Chlorpromazine in intravenous doses of 1 mg/kg significantly enhanced reticular inhibition of auditory input. In contrast, 10 mg/kg of pentobarbital depressed the effectiveness of the reticular formation in inhibiting sensory input.

Chlorpromazine has several important actions on the descending motor influences of the brainstem reticular formation. Its effects on the chemoreceptor trigger zone, in which it acts to prevent apomorphine emesis, are well known. Dasgupta and Werner (1954) have shown that chlorpromazine depresses the medullary vasomotor pressor response in intact chloralose-anesthetized cats. The effect is much more pronounced in decorticated animals. Gunn *et al.* (1955) observed a slight increase in the threshold for a pressor response to stimulation of the bulbar reticular formation and the splanchnic nerve. In many of these studies the role of peripheral ganglionic or adrenergic blocking effects has not been adequately evaluated. Martin and Eades (1960) compared the effects of chlorpromazine with those of atropine, pentobarbital, and chlorpromazine sulfoxide on vasomotor and EEG activation responses to stimulation of the midbrain reticular formation. They were able to show that both chlorpromazine and chlorpromazine sulfoxide decreased the excitability of the descending vasomotor as well as the ascending activating system. More recently, Wang *et al.* (1964) have shown that chlorpromazine depresses vasomotor areas of the brain. They used a crossed circulation experiment in which chlorpromazine was administered to the head of an animal; the head was connected to the body only by the spinal cord. Blood pressure in the body decreased even though chlorpromazine was administered only to the head. Thus, these investigators provided direct evidence that chlorpromazine depresses the central vasomotor regulatory system directly, which is consistent with the observations of others using less elegant techniques.

Chlorpromazine has definite effects on skeletal muscle tone. Henatsch and Ingvar (1956) have shown that chlorpromazine in doses of 0.5 to 2.5 mg/kg given intravenously to cats depresses *gamma* motor systems more selectively than *alpha* motor systems. Electrical stimulation of the globus pallidus or ventrolateral thalamic nuclei causes a facilitation of *alpha* motor neuron discharge. Chlorpromazine in intravenous doses of 1.5 to 5 mg/kg markedly augments *alpha* motor neuron discharge, while barbiturates depress it (Stern and Ward, 1962). The descending motor influences of chlorpromazine are particularly significant. Dasgupta and Werner (1955) have shown that chlorpromazine depressed the crossed extensor reflex of decerebrate cats; in spinal cats this effect was much less pronounced. This would indicate that chlorpromazine has both a brainstem and a spinal cord action. Krivoy (1957) demonstrated in intact and decerebrate cats that chlorpromazine in intravenous doses of 0.2 to 3 mg/kg significantly depressed mono- and polysynaptic potentials. On the other hand, in high spinal cat preparations, Preston (1956) reported that chlorpromazine even in large doses of 32 mg/kg intravenously had no appreciable effect on potentiated and unpotentiated mono- and polysynaptic reflex discharge. More recently, Hudson (1964) was able to show that chlorpromazine has a direct effect on the spinal cord of cats, affecting both facilitatory and inhibitory mechanisms. Various Russian investigators have shown that chlorpromazine has important effects on the reticular formation and spinal cord (Anokhin, 1957; Gvishiani, 1957; Khananashvili, 1957; Kreindler *et al.*, 1959; Kruglov, 1958; Valdman, 1962).

Most of the discrepancies in the literature on the effects of chlorpromazine on spinal reflexes are understandable in part on the basis of the preparations studied. In intact unanesthetized cat preparations Takaori (1958) showed that chlorpromazine in intravenous doses of 2 mg/kg depressed polysynaptic discharges. However, in spinal cat preparations sectioned at T2 chlorpromazine had no significant effect. Silvestrini and Maffii (1959) studied a series of phenothiazines, morphine, reserpine, and hydroxyzine on the patellar (monosynaptic) and crossed extensor and linguomandibular (polysynaptic) reflexes in cats and rabbits during and after chloralose or urethane anesthesia. They were able to show that small to large doses of chlorpromazine, promazine, reserpine, and hydroxyzine selectively depressed the patellar reflex of intact animals; the polysynaptic reflexes were much less affected. In spinal animals the depression of the patellar reflex disappeared. On the other hand, morphine and diethazine selectively depressed the polysynaptic reflexes more than the monosynaptic reflex. This effect was still present in high spinal animals. Hudson and Domino (1963) have shown that chlorpromazine has a significant depressant effect on bulbar facilitatory areas more than on bulbar inhibitory areas. This effect is not related to potentiation of anesthesia or hypotension, etc., but represents a direct action of the drug *per se.* The amount of background excitability in the spinal preparations considerably affects the degree to which chlorpromazine can be shown to have an action. Frequently, this crucial point is not taken into account and the result is considerable disagreement among various investigators.

SUMMARY

A review of the neuropharmacological literature reveals that the psychosedative drugs have multiple sites of action in the central nervous system. Despite the use of many different animal species and varying drug dosages, the data are remarkably consistent. Obviously any concept of site of drug action based only upon gross morphological areas of the nervous system is fraught with error. This has been elaborated in the Introduction with regard to the marked differences in drug effects upon various elements of the brainstem reticular formation. A functional scheme of site of drug action must be developed. Differences in site of action (such as between ethyl alcohol and barbiturates) may be marked. Even a single chemical family such as the barbiturates may show marked neuronal differences. For example, phenobarbital has anticonvulsant properties in doses which do not markedly depress the brainstem activating system, in contrast to pentobarbital. On the other hand, pentobarbital is much more euphorogenic than phenobarbital. Although the neuronal mechanisms for this are not understood, they probably will account for the fact that addiction to pentobarbital is much more prevalent.

To present an overall view of site of drug action which is generally applicable to most species, effects must be related to the minimal and to the maximal effective dose, but below a toxic or lethal level. Concepts of site of action unrelated to the dose administered are meaningless. Table 1 (p.61) lists various psychosedatives and some of their more important sites of action in the brain. The basal ganglia and cerebellum have been omitted because of a relative lack of data on the effects of these drugs on such structures. An arrow pointing upward means 'stimulation' of the neuronal area involved and an arrow pointing downward 'depression.' Although widely used, the words 'stimulation' and 'depression' are obviously very poor, for the ultimate neuronal mechanisms do not necessarily parallel these terms. For example, the primary mechanism of action of strychnine is to block postsynaptic inhibition. The stimulation which results is due to release from inhibition. As used in this review, 'stimulation' or 'depression' provide only a gross description of the drug's effect on an aggregation of neurons in a given area. Rather than simply stating that the drug acts on the brain, these terms may suggest what is going on at a less gross level of neuronal organization.

In a number of instances only indirect data are available from which to draw conclusions. This is especially true of the benzodiazepines, meprobamate, and to a lesser extent chlorpromazine. For example, the fact that meprobamate and the benzodiazepines produce 'fast waves' in the spontaneous EEG has been interpreted as evidence for slight depression.

Even a cursory examination of Table 1 supports earlier suggestions that these drugs do indeed differ in their sites of action in the central nervous system.

DISCUSSION OF DR. DOMINO'S PAPER

Introduction

Dr. Domino: The foregoing survey is based upon one that I made some years ago. That paper (Domino, 1962), dealt with the sites of action of some central nervous system depressants, but did not take up the question of ethyl alcohol, one of the principal drugs with which we are dealing here today. On finishing that survey I came to the conclusion that there was considerable chaos in the field. As a pharmacologist using the techniques of neurophysiology, I had great difficulty in trying to conclude precisely where any drug acts in the central nervous system.

TABLE 1

Sites of Action of Some Central Nervous System Depressants

Site	Association Cerebral Cortex	Systems Involving Thalamus Specific Relay	Diffusely Projecting	Hypothalamus	Limbic System	Brainstem Activating System	Spinal Cord Monosynaptic	Polysynaptic
Function	Association and Integration	Sensory	Arousal-Sleep	Emotional Reactions	Emotional and Memory	Arousal	Tendon Reflexes	Flexor Reflexes, etc.
Alcohol								
ED min.	sl ↑↓	sl ↑		sl ↓↑	?	–	sl ↓	↓
ED max.	↓↓	↓	↓	↓	↓	↓	↓↓	↓↓
Barbiturates								
ED min.	sl ↑↓	↓	↑ (I)	↓	↓	↓	–	↓
ED max.	↓↓	↓↓	↓	↓↓	↓↓	↓↓	↓↓	↓↓
Benzodiazepines								
ED min.	–	?	?	sl ↓	↓	–	–	↓
ED max.	sl ↓	?	?	↓	↓↓	↓	↓	↓↓
Meprobamate								
ED min.	sl ↓	↓	–, ↓(I)	↓	↓	–	–	↓
ED max.	↓	↓↓	?	↓↓	↓↓	↓	↓	↓↓
Chlorpromazine								
ED min.	–	–	–	↓	–, ↓	–, sl ↓	–	↓ (I)
ED max.	sl↓↑(I)	sl ↓	sl ↓	↓↓	↑↓	↓	↓ (I)	↓

Symbols:

ED min. – Minimal effective dose
ED max. – Maximal effective dose
(but below toxic or lethal level)
sl. ↑ – Slight stimulation
↑ – Stimulation

sl. ↓ – Slight depression
↓ – Depression
↓↓ – Marked Depression
(I) – Indirect due to an action at some other area of the CNS

Methodological Problems

Our interest in drugs that affect the nervous system stems primarily from their human application, and our main goal is to understand where drugs are acting in man. Yet all of us appreciate the fact that we must go to isolated neuronal systems, or other common models such as lower animals, in an attempt to understand how these drugs act. However, the very use of simple animal models makes it extremely difficult to extrapolate to the human situation, or from one animal species to another. Even within a species there may be considerable differences. These problems are further compounded by an increase in brain complexity among higher species. As a result it is very difficult to say much about the anatomical sites of action of different drugs in man. Generalizations are often made on the basis of data obtained in one species and subsequently assumed to apply to others. Yet, all of us can think of rather considerable differences in regard to drug effects in different species. The actions of various biogenic amines and their interaction with monoamine oxidase inhibitors illustrate these species differences very well.

As Dr. Russell has already pointed out, there are problems related to drug parameters such as the dose, and the duration of therapy. It is obvious that if we concentrate on problems of drug addiction, we shoul be thinking in terms of chronic drug effects and not those obtained on single administration. The pharmacological situation surely is quite different; for example, there is no opportunity for enzyme inductive effects to take place in the span of a few minutes or hours, but such effects might take place as a result of chronic administration of a drug. Also, one is concerned with the problem of the proper dose. For example, should we study barbiturates at a dose that causes a drunken state, or one that causes anesthesia, or in doses that cause behavioural but little or no neurological deficits?

I would like to raise yet another problem in interpreting the results of studies on the sites of drug action. Many neuropharmacological studies are performed in immobilized preparations, and the findings are not always valid for intact organisms. Some of these problems have been solved, or at least reduced considerably, by the use of chronic indwelling brain electrodes. This eliminates interaction with anesthetic and other rather gross disturbances to the physiology of the nervous system, but even with chronic implantation techniques we must bear in mind the role of possible glial scars at the electrode tips, etc.

Finally, we have to consider the difficulties of quantification of the phenomena that we record. Only in restricted cases, such as the spinal cord and a few other sites, where input-output relationship can be studied, is it becoming possible to identify the single units recorded.

Interpretation of Evoked Potentials

Many of our present hypotheses are indeed quite crude. Consider, for example, the problem of evoked potential recordings. Years ago it seemed a very simple matter to put an electrode in the reticular core and record an evoked potential. If the reticular response to an afferent input was depressed, we concluded that this represents neuronal depression, possibly at this site, or at least of the input to this site. It is obvious, however, that some stimulant drugs such as amphetamine, by causing an increase in neuronal discharge, reduce the amount of neuronal synchrony and, as a result, may reduce the amplitude of an evoked potential through an entirely different mechanism than neuronal depression. The opposite effect may be obtained with some 'depressants', such as morphine and chlorpromazine. If techniques such as this are used alone, it becomes extremely difficult to say whether the observed result indicates stimulation or depression of neuronal activity, if even these terms are really suitable.

Reticular Formation

The reticular formation provides a good example of the difficulties that one faces in attempting to determine where a drug acts. There is considerable disagreement as to the exact anatomical limits of this particular area of the brain, and within this complex there are perhaps a hundred or more different functional systems. Many of these are extremely important functionally, yet they cannot really be distinguished anatomically or histologically.

Consider some of the descending influences of the reticular formation, such as modulating those neurons involved in the facilitation and inhibition of motor activity at the spinal cord level. You can see that to talk about drug effects on the reticular core may be a gross oversimplification. The Russian pharmacologist, Valdman, has pointed out very well the limitation of the concept that chlorpromazine depresses bulbar facilitatory neurons and thus reduces higher centre influences on the gamma motor system. He was able to show that, at one discrete point in the reticular core, chlorpromazine had a pronounced depressant effect on bulbar facilitation of motor reflexes; and on another point, a few millimeters away, at which he also got facilitatory effects, chlorpromazine had no particular effect. He tried, with some success, to correlate this variability with some of the morphological characteristics of the reticular core. He concluded that there is considerable individual variation in sensitivity of these areas to the actions of chlorpromazine within the space of a few millimeters of reticular core.

Dr. Russell: Would another example of this be the observation reported by Grossman (1962), that adrenergic or cholinergic stimulation in the same CNS sites, in the same animal, produced quite different behavioural outputs? These results suggest specific neurochemical codings of functional neural circuits involved in at least certain forms of behaviour. Such coding would be important in differentiating circuits packed together as closely as they are in, say, the hypothalamus.

Multiple Sites of Action

Dr. Domino: That is a good point. In other words, within a given small area, one can have many neurons that respond in entirely different ways. Another and perhaps more classic example is the effect of CO_2. In small concentrations CO_2 stimulates neurons at a variety of levels, but neurons of the respiratory areas are exquisitely sensitive. Further, most drugs act at a series of levels in the central nervous system as well as peripherally. Consider, again, chlorpromazine. It has marked peripheral autonomic effects which may very well contribute to its overall behavioural effects. It also has important actions on skeletal motor reflexes. Several years ago, we thought that chlorpromazine had no significant effect on the spinal cord, and that most of its actions were on the bulbar facilitatory area, and action here would tend to depress the spinal cord reflexes. And yet, as our techniques have become more sophisticated, it has been demonstrated that chlorpromazine does have actions on the spinal cord.

Dr. Himwich: Are the concentrations that act on the cord the same as those required for the suprabulbar effects?

Dr. Domino: It appears to take a *little* more to cause this depressant effect in the spinal cord than it does in an intact animal. The point that Dr. Himwich has raised is a crucial one. When we talk about sites of drug action, we must keep in mind the dose that is administered. For example, if you look under 'Spinal Cord' in Table 1 (p. 61) you will see that I have indicated that chlorpromazine, at the 'minimal effective dose', does not affect monosynaptic reflexes; but polysynaptic reflexes are depressed, as indicated by an arrow pointing downward. The letter 'I' in parentheses indicates an indirect action because, in this case, its effect is on the bulbar facilitatory areas of the reticular core. At the 'maximal effective dose', the depressant effect on polysynaptic transmission is quite clear and there is also direct action on spinal cord levels. So the concept of dose, like that of concentration for our biochemical colleagues, is an extremely important one.

I think it would be hard to defend any exact definition of what I mean by minimal and maximal effective dose, but I believe you can appreciate the concept.

Dr. Brazier: What do you mean by the minimal effective dose?

Minimal Effective Dose

Dr. Domino: The smallest dose that produces minimal detectable neurologic or behavioural deficit. We have got to be careful because one can distinguish between these two measures, though in the case of chlorpromazine, I am not quite sure what is the minimal behavioural and what is the minimal neurologic dose for all species. I think the idea is fundamentally sound, that the minimal effective dose is one that has minimal effects in terms of neurologic deficit, and probably has some behavioural effects as well.

Dr. Brazier: What species is this?

Dr. Domino: I have attempted to generalize for all mammals; here again, one can criticize my approach. One can certainly think of some specific statements which do not apply to one species or another. Nevertheless, it is an attempt to provide a synthesis that will apply to most species.

In any event, it is clear that, to a large extent, drugs that affect the nervous system act at many different levels. As a result, such drugs are perhaps rightly considered by our neurophysiologist friends as essentially dirty tools. The majority of neurophysiologists have long believed that they should not depend solely on drugs to clarify matters of neuronal function; instead they use largely other physiological methods for proving their point. If drugs then happen to provide additional evidence to support their hypotheses, they will of course use them.

Maximal Effective Dose Versus Toxic Dose

Dr. Kalant: What is your concept of the difference between a maximum effective dose and a toxic one? Does it imply a difference in type of action?

Dr. Domino: No, not necessarily. Some drugs, such as digitalis, are effective when they are toxic, if you follow my thinking. By 'toxic' in a behavioural sense I mean a non-specific near-lethal action. In the case of barbiturates, a maximal effective dose is an anesthetic dose, but a toxic dose is one that stops respiration.

I would like to come back to the core of the argument that we are in a rather sad state of affairs with regard to trying to explain where specific drugs act. Perhaps the greatest contribution that Eccles and his associates have made has been to study discrete neuronal systems of known input and output so that one can talk about drug effects at specific neurons and synapses.

Lack of Comprehensive Theories of Drug Action

Grundfest (1964) has criticized neuropharmacologists for being weak in theory. He is correct in saying that most of us are preoccupied exclusively with collecting and reporting data, and to a large extent shy away from formulating or discussing theories of drug action. Central nervous system pharmacology is almost devoid of comprehensive theories of drug action. This lack might be related to the feeling that many of us have, that the present state of the science or art prohibits any but the most naive kinds of theories. Yet we all agree that theories are essential, for they suggest new and unique experiments, and allow generalizations from new facts, which makes for further progress. Perhaps the time has come for further and more elaborate electrophysiologic theories. The approach of Grundfest and Purpura and others (see Grundfest, 1964), even if sometimes difficult to follow by the less perceptive neuropharmacologist, nevertheless serves to emphasize the direction in which we must go.

Dr. Russell referred earlier to the fact that there must be a finite number of behavioural patterns that an organism can have, and that drugs can only modify these available patterns of behaviour. Similarly, one can suggest that there is a finite number of possible functional systems in the nervous system, and drugs differ only in the patterns of changes which they produce among these functional systems. Many drugs have a specificity of pattern of action at many levels in the central nervous system.

Barbiturates Differ in Action

One of the interests of this conference is in the comparison of ethyl alcohol with the barbiturates; let us begin by comparing their sites of action. You have to specify which barbiturates you are talking about, since phenobarbital and pentobarbital, for example, can produce quite different effects. For instance, phenobarbital depresses motor cortical thresholds in doses that do not have a significant depressing effect on the brainstem activating system. On the other hand, pentobarbital markedly raises the thresholds for both the motor cortex and the brainstem activating system, indicating that even within the same class of drugs there is some individual specificity of action. To group all barbiturates together, therefore, is certainly erroneous.

Ethanol and Euphorigenic Barbiturates

In comparing the actions of ethyl alcohol with those of pentobarbital, secobarbital, or any of the more euphorigenic barbiturates that are known to produce psychic and physical dependence in man, one notes that these compounds, which have many similarities in their behavioural effects, have quite similar neuropharmacologic effects at many different levels of the central nervous system. It seems to be largely a matter of individual preference, whether one stresses the similarities or the differences. In Table 1 (p. 61) I have listed a depressant effect as the primary action of ethyl alcohol and of these barbiturates on the cerebral cortex. Yet there is evidence that small doses of ethyl alcohol can actually lower the threshold for motor effects, at least for electrical stimulation of the motor cortex. Some people such as Masserman have interpreted this as evidence of a slight stimulant effect on these structures. Similarly, with the barbiturates, there is a seizure-facilitating action at certain small doses, but when massive doses are given the effect is primarily depressant. Thus, we talk of a mixed stimulant-depressant effect of these drugs. There are also similarities and differences in action at the level of the hypothalamus, the limbic system, and the brainstem activating system. Ethyl alcohol and the barbiturates show some differences in their spinal cord actions. Results of experiments done by us years ago indicated that in low doses alcohol *resembled* these barbiturates in having a slight selective depressant effect on polysynaptic as opposed to monosynaptic reflexes. Others who have studied this problem in more detail have tended to stress the *differences* between ethyl alcohol and the barbiturates. For instance, ethyl alcohol depresses mono- and polysynaptic pathways in the spinal cord at pretty much the same dose level.* In contrast, with barbiturates, at least in low dosage, one can get a depression of polysynaptic pathways more readily than of monosynaptic pathways. In the table, therefore, I have shown that in minimal doses barbiturates do not depress the monosynaptic pathways, while they do depress the polysynaptics, and that ethyl alcohol is less specific in this regard. With larger doses the barbiturates and ethanol do pretty much the same thing.

In considering compounds such as meprobamate, which are pharmacologically related to the barbiturates, one can again emphasize either the similarities or the differences. Certainly meprobamate is more similar in its spectrum of effects to the barbiturates than it is to compounds such as chlorpromazine. It would appear that from the point of view of site of action, alcohol, barbiturates, benzodiazepines and meprobamate cluster at one end of a spectrum, while drugs such as chlorpromazine are at the other end.

Iminodibenzyl Compounds

Dr. Himwich: What about the spectrum of the iminodibenzyls, for example, amitriptylene . . . . they're closer to which of the two categories?

Dr. Domino: That's a good question, Dr. Himwich. Had I not read the paper of Overall and his colleagues (Overall *et al.*, 1964), I certainly would have thought of amitriptyline and imipramine as being in a unique category. But it appears that imipramine, for example, is just as good an antihallucinant as is thioridazine, and thioridazine has an antidepressant effect similar to that of imipramine. This is a difficult area. I think we ought to place the iminodibenzyls in another class, on the basis of their prevention of uptake of catecholamines, even though they have spectra which overlap those of the phenothiazine-like drugs.

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*Ed. Note: See footnote on p. 40.

Commentary on Terms 'stimulant' and 'depressant'

One point that I would like to make is that the words 'stimulant' and 'depressant' are lay terms that have little precise pharmacologic meaning, though in the gross clinical sense, they may have some legitimate connotation. Yet there are gross inconsistencies in the use of the terms even clinically. For example amphetamine, commonly considered a 'stimulant', is used to normalize the behaviour of hyperkinetic children. On the synaptic level strychnine acts primarily to block the inhibitory transmitter for post-synaptic inhibition. Here is a substance whose basic action is depressant, yet which obviously produces intense stimulation of motor reflexes. The barbiturates are 'depressants'; and yet they have a euphorigenic action. Is that depression? We use these terms and yet we would have a great deal of difficulty in defining them.

Dr. Dews: Why not eschew these terms altogether and use instead words like 'reduction' and 'increase.' The advantage of this is that if people say something is an increaser, they are very likely to say what the something is that is increased; when people say that a drug is a stimulant, they feel as though they have said something of meaning, and may stop there without saying what is stimulated. Yet 'stimulant' means no more than 'increaser.'

Dr. Hoff: In that connection, I wonder what were the functions actually referred to in your table, under 'hypothalamus' and 'limbic system', which you designate as emotional reactions and memory. Were these stimulated or depressed?

Dr. Domino: In deciding where these drugs were acting, I considered such things as afterdischarge in the hippocampus. You are probably wondering what this has to do with memory?

Dr. Hoff: Yes, that is right. What do you actually mean by emotional reaction?

Dr. Domino: I mean it in terms of the accepted view that the hypothalamus is one of the main effector mechanisms of various kinds of emotional behaviour. Perhaps it would be much better to delete the whole line indicated as 'Functions', because it gets us into the difficult question of correlating function with anatomical site of action.

Dr. Hoff: I'm glad you specified that, because I was wondering whether you meant that you were actually testing signs of emotional reaction . . . .

Dr. Domino: Masserman did test the effects of ethyl alcohol and barbiturates on emotional behaviour. But in other work, classic neurophysiologic end-points were used.

Dr. Kalant: When you talk of stimulation or depression of, for example, the association cortex, are you referring exclusively to spontaneous activity, or to direct local excitation threshold, or to evoked responses?

Dr. Domino: In the case of ethyl alcohol, Masserman (1940) stimulated motor cortex as well as some of the premotor areas, and showed a change in threshold. That is why that particular area is in the table. In that case we should be talking about motor cortex and not association cortex.

Dr. Russell: One of the problems we always run into here is the difficulty in shifting from one level of discourse to another; from the level of, say, electrophysiological measures to behavioural measures. After administration of amphetamine, for example, certain forms of behaviour will be enhanced. Other forms of behaviour, that are dependent upon repression of certain aspects of responding, are definitely impaired under these same circumstances. The vagueness of terminology is just as discouraging in behavioural as in neuropharmacological studies.

Dr. Domino: Dr. Dews, you suggest dropping the terms 'stimulant' and 'depressant'. What would you substitute for them?

Dr. Dews: You could talk of 'barbiturates and similar compounds', 'amphetamines and similar compounds', and so on down the list.

Dr. Domino: How would you classify antidepressants? Are they stimulants or depressants? They really are both!

Dr. Himwich: Clinicians use them to treat a target symptom.

Alternative Bases for Drug Classification

Dr. Domino: Perhaps our solution should be to classify drugs on the basis of their use for treatment of target symptoms, and then wait until the body of knowledge grows enough in the next couple of thousand years to let us say whether they are stimulants or depressants, and of which specific neurones. I'm sure that some of our biochemical colleagues will argue that there is no such thing as a drug being a stimulant at a biochemical level. Usually one conjectures that there is a second reaction depressing a first reaction, and drug suppression of the second reaction now allows the first to proceed more rapidly. The 'stimulation' caused by the drug is explained ultimately as a blockade of depression of biochemical activity.

Dr. Kalant: This is Albert's concept of selective toxicity (Albert, 1965) – that any drug acts to *inhibit* some reaction. In his view there is no such thing as a specific stimulation of a reaction by drugs. But is this of any help? Is there enough knowledge to permit the classification of drugs in terms of their functional effects even in specified cells, on the basis of what type of reaction they are inhibiting?

Dr. Domino: I like the idea of a physiological approach. In fact Dr. Brazier (1954) has used this to a certain extent in relation to anesthesia, and has talked in terms of anesthetic agents affecting afferent inputs, integration, memory mechanisms, etc. Perhaps this kind of approach is the most fruitful.

Co-enzymes as Stimulants

Dr. McIlwain: Isn't it true that most drugs which have a highly stimulant effect produce it by actually releasing mechanisms which normally are under control? This is certainly involved at biochemical levels. Yet in viewing biochemical agents as a whole, there most certainly are stimulants, as co-enzymes are stimulants . . . .

Dr. Brazier: Stimulants to what, as Dr. Dews would say?

Dr. McIlwain: As co-enzyme I is a stimulant to lactic dehydrogenase.

Dr. Brazier: Is that to a chain of chemical reactions?

Dr. McIlwain: To an individual chemical step.

Dr. Brazier: A trigger for a chemical chain?

Dr. Himwich: A trigger for an energy-releasing process will do this.

Dr. Quastel: But that's probably because it's inhibiting something else.

Dr. Domino: Don't the thiobarbiturates act as stimulants to oxygen consumption at just the right dose to act as an uncoupling agent?

Dr. Quastel: Yes, they are stimulators in that sense, but because they inhibit something else, in the same way as dinitrophenol.

Dr. Dews: What about the effects of dinitrophenol on lysosomal hydrolysis?

Dr. McIlwain: This happens to be a release of something which has been membrane-bound (Weissman, 1965).

Dr. Brazier: May I ask what you mean by membrane-bound?

Dr. McIlwain: There has previously been a hydrolytic enzyme within a particular subcellular structure; if this structure is broken down, then the enzymes can come into action.

Dr. Brazier: You mean in the neuronal membrane? Glial membranes?

Dr. McIlwain: No, I'm thinking of subcellular particles, the lysosomes, but fundamentally I don't imagine this is very different from the situation in activation of acetylcholine hydrolysis.

Ethanol, Axonal Conduction, and Synaptic Transmission

Dr. Domino: I'd like to point out, in relation to a specific drug, some of our problems with the term 'membrane.' Our biochemical colleagues tend to use it in a more general sense. Consider the actions of ethyl alcohol on the neuronal membrane. Earlier, Dr. Wallgren and I were discussing the observations by Larrabee and Posternak (1952). They isolated the superior cervical ganglion of the cat, stimulated it preganglionically, and recorded the responses both in the direct axonally conducting fibres and in the post-synaptic fibres emerging from the ganglion. In our talk, Dr. Wallgren and I referred to ethyl alcohol and its mechanism of action, and the term 'membrane depressant' came up. I pointed out that Larrabee's work showed the synapse to be the critical site of action of many drugs, including long-chain alcohols, but that the short-chain alcohols resembled urethane in depressing axonal conduction at much lower concentrations than those needed to depress synaptic transmission. In fact, ethyl alcohol actually had a synaptic facilitating effect in contrast to a blocking effect.

Dr. Wallgren: It appeared that the non-synaptic pathway was actually even more sensitive to ethyl alcohol than the synaptic pathway. The preparation was the cat stellate ganglion. Stimulating electrodes were placed on myelinated preganglionic fibres, which were either presynaptic or passing through the ganglion without synaptic contact. Recording was from unmyelinated post-synaptic fibres (inferior cardiac nerve) to measure 'transmission', and from myelinated direct fibres (cervical sympathetic trunk) to measure 'conduction.' However, no attempt was made to control for differences in the susceptibility of the fibres as such.

I think it is permissible to conclude that if you have, at clearly lower alcohol concentration, a block in the pathway which includes synaptic transmission, then it is very likely that the drug has a selective action on synapses. However, it's not entirely certain, if you don't record very closely presynaptically also. I know they haven't looked at the axon.

Dr. Himwich: Which depression appeared first at the lowest dose of ethanol?

Dr. Wallgren: The non-synaptic. . . .

Dr. Himwich: When they increased the doses, was the synaptic pathway also inhibited?

Dr. Wallgren: They came to a concentration at which conduction was blocked in both these pathways. But a 50% decrease in magnitude of the compound action potential in the direct fibre was produced by 250 mM ethanol, while the same degree of depression in the synaptic pathway required 480 mM. When they used butanol, there was no difference between the effects on the two pathways and with higher alcohols the synaptic pathway was more sensitive.

Dr. Himwich: Would that parallel the numerous observations that low doses of ethanol are frequently stimulatory and higher doses are more depressant? In other words, possibly you might even have some facilitatory effect on transmission at lower doses?

Dr. Wallgren: Oh yes, that is a biphasic effect that is correlated with the depolarizing action. With a slight depolarization you get a lower threshold, and that applies at all levels of integration, and pertains also to synaptic transmission. When the same concentration of alcohol gives facilitation of some excitable preparations and inhibition of others, it's a question not so much of different mechanisms of action as of different dose-response relationships.

Dr. Domino: As I understand them, the Larrabee-Posternak findings indicate that sparing of transmission across the synapse under the influence of ethanol was due to a partial masking of depression by a concurrent facilitation at the synapse.

Dr. Wallgren: This is one interpretation given by the authors. They also suggested, among other possible explanations, that the fibres traversing the ganglion are more sensitive than any part of the synaptic pathway is, to agents such as ethanol.

Dr. Dews: You don't actually need to postulate a facilitation of transmission across the synapse to account for the effects with low doses of alcohol. You could imagine that if the size of impulse reaching the synapse declines by less than a certain amount, it is still suprathreshold for producing the full reaction of the post-synaptic membrane. You referred to the experiments of Masserman on the cortex. These are, I take it, the experiments done 25 years ago?

Ethanol and EEG Activity

Dr. Domino: Yes. More recent work is by Story, Eidelberg and French (1961). They compared the effects of ethyl alcohol at various dose levels in cats with indwelling brain electrodes, and correlated the changes in electrical activity with blood alcohol levels. They showed that intravenous and intraperitoneal administration of alcohol gave pretty much the same blood level. There was a slight difference, as one would expect, a slightly higher level early with the intravenous injection, but with doses in the order of 2 ml/kg the blood levels were the same whether the drug was given intraperitoneally or intravenously.

Dr. Kalant: Yes. Absorption of ethanol from the peritoneal cavity is almost immediate (Czaja and Kalant, 1961).

Dr. Domino: Excellent! In addition, they measured the EEG activity in the various neocortical, thalamic, limbic and reticular areas, and compared this to the behaviour of the cats in the awakened state.

Essentially they showed that ethyl alcohol in doses that produced a light drunkenness did not really produce any significant EEG changes. The changes that occurred were similar to those found under natural conditions without any ethyl alcohol. This agrees with many reports that alcohol differs from the barbiturates in that it doesn't produce 'fast waves' in the EEG. I don't know whether this should let you conclude that there is no cortical effect of ethanol, since the EEG is rather gross. In fact, Dr. Brazier, I don't know what EEG 'fast waves' mean. Is this a sign of neuronal stimulation or depression, if we should even be using these terms?

Dr. Brazier: I wish we knew.

Ethanol, Direct and Cortical Recruiting Responses

Dr. Domino: To continue discussing the work of Story *et al.*: if you stimulate the cerebral cortex you get an evoked potential which is called a direct cortical response. Purpura and Grundfest suggest that synapses are involved, and that this potential is a summation of excitatory post-synaptic potentials (EPSP's) and inhibitory post-synaptic potentials (IPSP's), (Grundfest, 1964). After ethyl alcohol the so-called direct cortical response is slightly enhanced and after a dose of ethyl alcohol that produces drunkenness, the transcallosal and inter-hippocampal responses are also enhanced. In contrast, cortical recruiting responses produced via diffusely projecting thalamic nuclei are *not* affected, but the responses to stimulation of the specific relay nuclei, including the somesthetic as well as the visual system, are very markedly depressed. Story *et al.* have postulated that alcohol acts in these areas to depress cortical interneurons of the Golgi type II variety. Here is a very clear-cut hypothesis that someone could perhaps test by isolating Golgi type II interneurons and showing that they are indeed exquisitely sensitive to alcohol. This occurs with doses of ethyl alcohol that produce only a mildly drunken state; so we say there is some good evidence for a cortical depressant effect of alcohol. On the other hand Kolmodin (1953) has shown that mono- and poly-synaptic pathways in spinal animals or decapitated animals are depressed, indicating that ethyl alcohol acts at spinal cord levels as well.

Barbiturates and Presynaptic Inhibition

Neurophysiologists such as Eccles (1963) have obtained evidence that barbiturates *prolong* presynaptic inhibition and have postulated various mechanisms by which this occurs. One possibility is that barbiturates inhibit the action of an enzyme that destroys the transmitter substance for presynaptic inhibition; another is that it somehow increases the barrier so that the neural transmitter doesn't diffuse away from the synaptic site as readily. One can use these suggestions to formulate an interesting theory of physical dependence on barbiturates. Let us accept that presynaptic inhibition is enhanced during barbiturate administration, due to decreased effectiveness of enzyme that normally destroys the inhibitory transmitter. The net result might be that in time the organism would create more of this particular enzyme, or decrease the amount of inhibitory transmitter that is formed or released. One would then predict that after a period of time, if one suddenly withdrew the barbiturate, the organism would be left with too much enzyme or not enough of the inhibitory transmitter to cause pre-synaptic inhibition, and therefore increased excitability of the central nervous system would result. This hypothesis may offer a challenge to our biochemical colleagues to find the inhibitory presynaptic transmitter or the enzyme that breaks it down, for this concept emphasizes that drugs may be highly selective in their actions and not affect every neuron equally. Should we not suggest to the biochemists that they might be more selective in the cell systems which they study chemically?

Development of Barbiturate Dependence – a Hypothesis

Dr. McIlwain: I think experiments such as you have suggested will be enlightening insofar as the biochemist has been guided to the right part of the brain by neurophysiology and neuropharmacology.

Dr. Domino: Part of my motivation for attempting to determine where drugs act in the central nervous system is exactly that: to be able to tell my chemical colleagues where to look. On the other hand, there may be some benefit in the fact that drugs *are* 'dirty tools', if the only way we can sharpen them up is to use the smallest effective concentration. Even though a drug may not be acting *in vivo*, for example, at a cortical neuron, the cortical neuron may still serve as a model. If in moderate concentrations the drug produces some effect on it, one hopes that at a lower concentration it will act similarly at some other site which one can identify.

Biochemical comment on barbiturates, tranquillizers and ethanol

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This account aims at following the Convener's suggestions in appraising chemical and metabolic aspects of the actions of tranquillizers, barbiturates and ethanol, and in seeking to discern what is not known as well as what is known. Comparisons among the compounds are made incidentally rather than within a formal scheme. There is, throughout, the aim of useful comment in relation to problems of addiction. Minimal comment is made on subjects likely to be appraised by other members of the Conference. Among synthetic drugs, the actions which guided selection and synthesis receive specific note, as they can be regarded as having shaped a drug but are not necessarily the same as the actions which give the main occasion for therapeutic use.

BARBITURATES

Origins. With the barbiturates, a highly evolved group of drugs, origins are very relevant to actions. In the first group of compounds, as has often been told, selection was on the basis of the duration and the speed of onset of sleep in experimental animals. In this and subsequent studies, some undesirable side effects were also recorded and contributed to selection, but little or no attention was paid to tendency to produce addiction or dependency. Scope therefore remains, at this initial level of investigation, for seeking to apply or devise tests of addictive tendency in such drugs and to incorporate them in screening programmes.

How far back in barbiturate development might such a reappraisal extend? Fischer and von Mering's compounds derived from the synthesis of substances cognate to uric acid; and from known central depressant actions in amides, ureas and urethanes, and their enhancement by ethyl substituents. Taking account of this, barbitone was possibly selected from a hundred or so compounds, and a more evolved drug, such as thiopentone, from a thousand or so analogues. This indicates the scale on which a test for addiction or dependency might need to be used. Attempting to select among a few already evolved drugs to find those of minimal addictive tendency is not a comparable procedure.

Barbiturate action. It is primarily physiological and pharmacological studies which have shown that the functions of a number of parts of the brain are depressed by particular barbiturates. Associated with the overt effects of light pentothal anesthesia in cats, Sokoloff (1961) found diminished blood flow in 14 out of 28 cerebral areas examined; these were all of grey matter, while flow in white matter regions was not significantly altered. Most affected were the primary sensory areas, in which flow was diminished by 40-50%. This represents an impressive localization of action in an anesthetic agent.

It is to be expected here that diminished blood flow implies diminished substrate utilization; this is certainly the case in the brain as a whole, as shown by oxygen and carbon dioxide measurements in arterial and cerebral venous blood. Nevertheless, energy-rich substances accumulate in the brain during barbiturate anesthesia (Stone, 1940; Lin *et al.*, 1968). The depression of function, when it could be examined in isolated neural systems, was greatest where cell-firing rather than conduction was involved (Larrabee & Horowicz, 1956).

With isolated cerebral tissues phenobarbitone at 0.3 – 1 mM had little action on respiration until the tissues were electrically stimulated, when respiration became lower in the presence of phenobarbitone but phosphocreatine concentration was higher. Again, therefore, inhibition of respiration in cerebral systems by a barbiturate appears secondary to inhibition of functional activity. Respiratory inhibition may play an auxiliary role in their action as drugs: possibly a balance between action on energy-yielding and energy-utilizing processes is needed for a successful anesthetic. This is, however, made less likely by the observation that thiobarbiturates can uncouple oxidative phosphorylation, while oxybarbiturates do not (Aldridge, 1962; McIlwain, 1962), yet their characteristics as drugs appear not to differ radically.

The speed of action of these two categories of barbiturate does differ, and this is correlated with their speed of distribution in the body. Distribution and metabolic change in certain barbiturates appear to limit barbiturate action without otherwise characterizing it. Tolerance to pentobarbitone induced in rats by repeated administration was associated with increased oxidation of the drug by liver preparations (Remmer, 1963).

Comment. Neurochemical studies probably still have much to contribute to an understanding of the actions of barbiturates. Knowledge of the structure of excitable membranes and of the reorganization which they undergo on cell discharge may reveal components or arrays analagous or complementary to the structure of barbiturates. If localized cerebral systems are specified as particularly associated with barbiturate action or addiction, chemical characteristics might be found in these particular parts of the brain which could contribute to the design of drugs. To judge by the existence of convulsant barbiturates, and by the antagonistic properties of bemegrid, much is yet to be learned of the specific structures with which barbiturates interact.

TRANQUILLIZERS: CHLORPROMAZINE AND RESERPINE

Among tranquillizers, comment is first made on chlorpromazine and analagous phenothiazines, with a few notes on reserpine; observation on γ-aminobutyrophenones follow.

Origins. Chlorpromazine was successfully designed by animal assay to have particular effects, but the effects examined were not closely related to its action as a tranquillizer. Assays again included measurement of sleep in experimental animals, but a test for antipyresis was also included in selection. With these two tests and a specific initial compound of phenothiazine structure, the substance evolved was very different from the barbiturates. Among the differences was an absence of tendency to induce addiction or dependency (Seevers and Deneau, 1963). This may offer a suggestion in designing other drugs lacking such tendency; the difference presumably depends either on the choice of initial compound or on the choice of animal assay.

The measurement made of sleeping-time in the development of phenothiazines was not a simple one but involved potentiation of the action of a low concentration of ether, which was given to mice and which did not itself inactivate the animals. The initial compounds from which chlorpromazine was developed were antihistamine drugs in which central actions had been found. These were first applied in a technique termed artificial hibernation designed to lower body temperature and autonomic response in shock or in certain surgical operations. This technique will be recognized as making quite direct application of the qualities selected by the assays which yielded chlorpromazine. The suggestion (Laborit *et al.*, 1952) that these qualities might also be useful in psychiatric conditions was made because treatments involving prolonged narcosis were already considered of value in such conditions. Phenothiazine sedation independently of ether or other general depressant thus became established, but by observing incidental virtues in a compound rather than by deliberately inducing them in assay.

In *Rauwolfia,* also, the analagous properties were observed and were reproduced by *Rauwolfia* components including reserpine; again, this compound does not appear to induce addiction but some of its side effects are unpleasant.

Actions. Body temperature can be so successfully manipulated by intraventricular serotonin and catecholamines (Feldberg and Myers, 1964) as to make it understandable that systems involving these compounds should be concerned in the actions of a drug selected by the tests which yielded chlorpromazine. The amines appear to be involved in fashions amenable first to pharmacological characterization and best appraised by others. Chlorpromazine has been suggested as acting by inhibiting the uptake of catecholamines or serotonin to their sites of cerebral storage, but this, in at least some circumstances, is secondary to the hypothermia caused by chlorpromazine; when the body temperature of the sedated animals was maintained, uptake of the amines was normal. On the other hand, chlorpromazine (10 mg/kg) has been found to minimize the loss of noradrenaline from the brainstem of rats, in response to stress (Maynert and Levi, 1964). In these experiments the stress consisted of electric shocks to the feet of rats from the floor of their cage; the loss of noradrenaline was blocked also by phenobarbitone (50-100 mg/kg) but not by morphine.

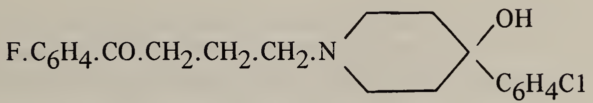
The action of chlorpromazine at the neural systems on which it acts can be described as causing diminished response to excitation. A primary metabolic site for its action as a tranquillizer has not been firmly established. An appraisal (McIlwain, 1962) of actions reported on cerebral respiration in a number of systems, on the major tissue phosphates, and on oxidative phosphorylation has indicated that these are unlikely to represent such primary sites of action. These characteristics measured in isolated cerebral tissues were not affected by 5-10 μM chlorpromazine until the tissues were electrically stimulated, when normal responses to excitation were diminished. Change in membrane potential, and movements of sodium and potassium ions on excitation, were also restricted by chlorpromazine. An action at systems involved in nerve-impulse initiation or spread is thus indicated. The action thus appears analagous to the effect of chlorpromazine in maintaining brainstem noradrenaline: here also the drug did not alter the normal level of noradrenaline, but prevented the diminution caused by stimulation.

In understanding why chlorpromazine acts at subcortical rather than cortical systems, observation on its differential localization are very relevant (de Jaramillo and Guth, 1963; Tadros and Wahab, 1962). Given intravenously to dogs and rabbits, and the brain sampled at intervals of 15-90 minutes afterwards, chlorpromazine was found at concentrations of 150-200 μg/g in the medulla, hypothalamus, basal ganglia and thalamus but at 0-10 μg/g in the temporal or frontal cortex. Prochlorperazine, also a phenothiazine tranquillizer but with a piperazine substituent, was similarly localized but thiethylperazine, structurally related but not a tranquillizer, was not. This compound had potent antinauseant, antivertigo and antiemetic properties and was especially concentrated in the cerebellum (300 μg/g), hippocampus and part of the thalamus (75-90 μg/g). Localization of chlorpromazine in the monkey in favour of the brainstem was also found.

The outstanding actions of reserpine in releasing serotonin and noradrenaline have been so well investigated by others here that I will not attempt to describe them. They appear to give an example in which the occurrence of these bases in particular parts of the brain has defined the areas susceptible to reserpine. Similarities between the sedation caused by reserpine and that caused by chlorpromazine appear to depend on the parts of the brain on which they act; these overlap but are not identical, as Dr. Domino has noted in more detail. Not all actions in reserpine-like compounds are, however, associated with liberation of catecholamines or serotonin (see Vogt, 1965) and much remains to be understood of their fundamental action in liberating the bases.

AMINOBUTYROPHENONES

Haloperidol is noteworthy as a depressant akin in several properties to chlorpromazine, but produced on the basis of animal assay among structural variants of meperidine: that is, from a compound of marked addictive properties. The assays which guided selection and synthesis included the potentiation of pentobarbitone hypnosis, the measurement of righting reflexes, of balance on a rotating rod, of reaction to a hot plate and mydriatic action (Janssen *et al.*, 1959; Janssen, 1964). Using these tests, depressant properties were found in a group of piperidinobutyrophenones which were similar in many respects to promazine, the most suitable, haloperidol, being



The tests employed made it possible to select compounds which were not analgesic, and also which did not show morphine-like excitement nor the Straub phenomenon. Potent analgesics were also developed from the same starting compounds but with different criteria of selection (Janssen, 1961).

In exploring these compounds metabolically, haloperidol, meperidine and also two other aminobutyrophenones were found to act on isolated cerebral tissues, diminishing tissue responses to electrical stimulation at concentrations of 10 μM or less (McIlwain, 1964a). In this system one characteristic was found in which haloperidol resembled chlorpromazine but differed from meperidine and the other two butyrophenones. This was in tissue content of sodium ions after electrical stimulation (Fig. 1).

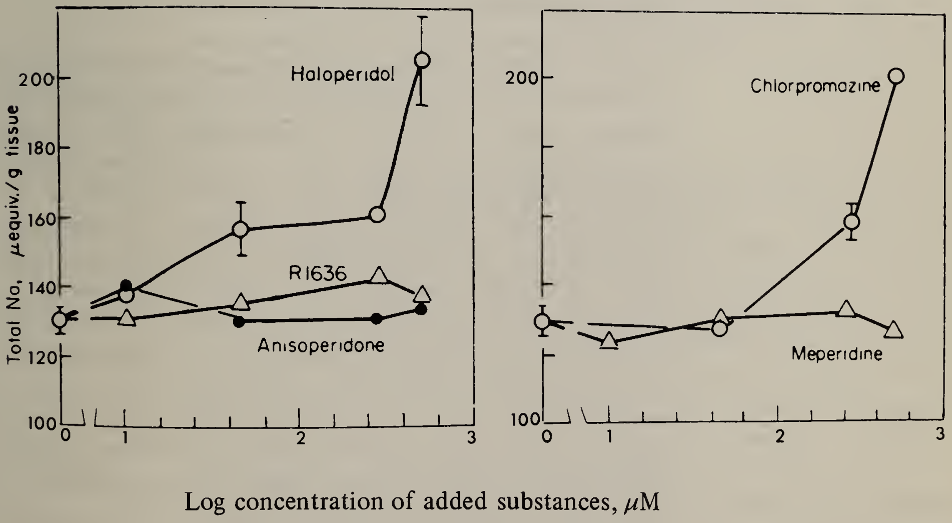


Figure 1

The sodium content of guinea pig cerebral cortical tissues after incubation and electrical stimulation in the presence of added substances. Reprinted from Biochem. Pharmacol. *13:* 523, 1964 with the kind permission of the publisher.

As this action required high concentrations of chlorpromazine and haloperidol it is not itself likely to be significant in their action as drugs, but represents a differentiating characteristic which may merit study in particular parts of the brain, as may also the localization of the aminobutyrophenones. Haloperidol is akin to chlorpromazine in allowing accumulation, in the brain of mice, of 3-methoxytyramine and methylnoradrenaline, which are normal catecholamine metabolites (Carlsson and Lindquist, 1963).

ETHANOL

Ethanol is of very different biological status from the preceding compounds; selected at a time when food and drugs were barely differentiated, it still falls into both categories. It is, moreover, present in normal animals to which it has not been administered, at some 30 μM (Lester, 1962)*; more primitive forms of life as well as more primitive societies include some which have been much exposed to ethanol.

General Metabolism.

The metabolism of ethanol in the body as a whole greatly conditions its effects in man: up to 400 ml of ethanol can be oxidized per day and make a major contribution to energy supply. When this quantity was first taken by volunteers in small, frequent doses it produced gross intoxication and blood levels of 30-50 mM ethanol. After 10-14 days with this dose and adequate dietary supplements, the concentration in the blood fell rapidly to zero (metabolic tolerance) with a concomitant disappearance of intoxicated behaviour. However, small increments in alcohol dosage sufficed to raise blood levels to between 30 and over 50 mM (approximately 150 to over 250 mg/100 ml) with re-induction of behavioural intoxication. Thereafter, concentrations of alcohol in the blood remained high, but toward the end of the longer intoxication periods, behaviour was less incoordinated and better controlled (tissue tolerance) (Wikler, 1960). Adaptation to ethanol had thus been made both by its increased metabolism and by some modification which permitted more normal cerebral functioning in its presence. Withdrawal of alcohol then produced a variety of physical and mental changes.

In rats, ethanol at about 0.6 g/kg increased the blood levels of acetaldehyde, acetates and acetoacetates, and the metabolites from ^{14}C-ethanol indicated metabolic routes through acetaldehyde and acetylcoenzyme A, with the liver as a major site of metabolism (Forsander and Räihä, 1960; Russel and van Bruggen, 1964; see Kalant, 1962). Some acetaldehyde reached the general circulation (0.1-0.2 mM) when blood ethanol was 20 mM in man (Sutherland *et al.*, 1960) and contributed to certain of the side-effects of ethanol. Endocrinological changes also occurred, which may be appraised by others.

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*Ed. Note: Walker and Curry (1966) found that amounts in blood and urine are less than 0.1 mg/100 ml (20 μM).

On administration, quantities of ethanol producing facial vasodilatation and mild inebriation have not been observed to produce changes in cerebral respiration or blood flow. There are perhaps just detectable diminutions at 20 mM when intoxication is evident (Hine *et al.*, 1952; Sutherland *et al.*, 1960); these results refer to the whole brain, in man. Cerebral cortical tissues from experimental animals show some cognate effects with ethanol levels of 60 mM or greater, respiration being slightly inhibited in electrically stimulated tissues. The most marked effect in these systems is on utilization of energy-rich phosphates: fall in phosphocreatine on stimulation was inhibited 50% by 87 mM-ethanol (Wallgren and Kulonen, 1960), as Dr. Wallgren himself will probably indicate. Other simple alcohols, at concentrations giving similar thermodynamic activities, gave similar inhibitions; in these respects the compounds appear to be acting as general depressants. Again, it is other workers here who can best indicate the parts of the brain most susceptible to ethanol action.

Mechanisms of Action

Ethanol, with other alcohols, has featured in many studies of the action of general depressants. These favour an action in common with surgical anesthetics and of one of the following types. (i) By their mere presence, they may act as diluents or contribute to a barrier at a structure or interface. Solution in or association with lipids of cell membranes may be involved, and result in diminished membrane permeability.* Skou (1961) suggested that penetration of lipid monolayers by alcohols or by local anesthetics might lead to diminished Na^+ entry during a nerve impulse, by such a mechanism. (ii) The depressants are also suggested as interacting with water to produce clathyrate compounds or labile microcrystalline association compounds (Pauling, 1961). It is pictured that the polar groups of proteins become involved in such associations and so become less available for their normal functioning (Featherstone, 1963). That this variety among theories remains, without characterization of the protein or lipid components believed to be involved, emphasizes the lack of fundamental knowledge at the centre of the subject of neurochemistry: of the nature of the membrane reorganization involved in initiation and spread of the nerve impulse.

*Ed. Note: Evidence in support of this hypothesis was later provided by Seeman (1966).

DISCUSSION

Drug Evolution and Selection

Dr. McIlwain: The process of evolving synthetic drugs such as chlorpromazine or barbiturates is a piece of applied biology that has quite definite rules to it, and these rules almost necessitate going through stages in which the specificity of the agents being involved is not high. Whether you stop at that stage or whether you continue to evolve the drug, is a definite choice on the part of either the investigator who determined the use of the drug, or of the people who use it. It is a decision which is made at different levels with respect to different drugs, but it is not something that is arbitrary or accidental. It is a human decision as to whether one is handling a drug of high specificity or of rather low specificity; but I think that all synthetic drugs have gone through a phase, during which many have been discarded, in which their specificity is low. The reason is that the process of making a synthetic drug is one which evolves something of greater specificity from something which previously had little specificity.

The question of how the drugs are evolved also answers at least one of the problems that was felt to be rather unsatisfactory in earlier discussion. This is that one investigates in experimental animals drugs that are to be used in human therapy. In fact, any synthetic drug which is being developed necessarily acts on a number of species including the experimental animals in which it was developed. This, I think, must always be remembered. If possible, one should remember which particular experimental animals were used during development of the drug, although in practice very often one forgets. For instance, with the barbiturates, there was much use of rabbits, while with chlorpromazine it was rats and mice. Actually species differences do exist; therefore it matters exactly what species have been examined. But the comment that we investigate, in experimental animals, drugs which act in humans, is only partly true. It definitely does not refer to any highly evolved synthetic drugs. So I tend to look at a drug as an achievement in producing a given type of action at a pharmacological or behavioural level. If in addition the drug has some fairly specific effects at biochemical levels, then one regards this as something which is very interesting and probably implies that one is obtaining a significant lead about the nature of the drug action.

Thiopentone and Cerebral Blood Flow

The data regarding blood flow, quoted in my paper, illustrate this with respect to a general depressant, thiopentone, which was designed to depress sensory stimuli. In fact it does have a major effect on quite specific regions of the brain, that is, on sensory regions of the cortex.

At the same time as blood flow is being depressed, oxygen uptake is being depressed in the brain as a whole. The oxygen uptake has not been examined in the same regional detail.

Dr. Dews: Are these measurements obtained by the nitrous oxide technique?

Dr. McIlwain: They are measurements by observing diffusion into the brain of radioactive trifluoiodomethane. This compound diffuses very readily from the blood stream, and after it has reached a given blood level, the experimental animals can be killed, and prepared. Subsequently the amounts of the compound in the different cerebral regions can be estimated from an autoradiograph, and the amount of the compound in any area at any particular stage gives an approximate measure of the blood flow.

Dr. Domino: Dr. McIlwain, one can perhaps conclude that in sensorimotor cortex the drug is reducing oxygen uptake by half. But in the reticular formation there is apparently not a statistically significant reduction in blood flow. I think this illustrates the kind of discrepancy that creates difficulties for the neuropharmacologist. One of the principal actions of the barbiturates is on the brainstem reticular formation.

Dr. Himwich: Perhaps the effect is not produced by a change in metabolism?

Dr. Domino: Maybe, but then why is the neocortex affected? Both structures are depressed by the barbiturate from a neuropharmacological point of view, and yet in the cortex there is a decrease in metabolism but in the reticular core there is not.

Dr. Himwich: Dr. McIlwain, aren't there similar biochemical studies of other drugs?

Control of Cellular Respiration

Dr. McIlwain: Not in this detail, that I am aware of. If one may proceed from this to some sort of biochemical analysis of what the mechanism may be, the data quoted indicate one stage; granted that there has been a diminution of brain tissue respiration, the next question is how that is mediated. The conclusion is that respiration is a process that is normally controlled, and part of the control mechanism is the availability of adenosine diphosphate and inorganic phosphate.

Dr. Domino: Does this mean that ATP synthesis is continuing in the presence of pentobarbital or allobarbital but utilization is not, and therefore one finds lowered inorganic phosphate levels?

Dr. McIlwain: Yes. The main sources of the inorganic phosphate are the energy-rich phosphates, such as adenosine triphosphate. When, in the presence of a depressant drug, one sees a smaller concentration of inorganic phosphate, one pictures that there has been a lesser utilization of adenosine triphosphate producing less inorganic phosphate and ADP. The reduced production, then, results in less respiration. This applies also to the action of chlorpromazine. Chlorpromazine was not derived in terms of what we now think of as a tranquillizer. It was devised as an agent which would modify ether anesthesia and which would modify body temperature. In considering its action, perhaps one could look at body temperature and its chemical control, which involve the hypothalamic regulatory centres and the action of catecholamines and serotonin on them. Administration of very small amounts of adrenaline or noradrenaline will depress body temperature of a variety of experimental animals; this depression will last for a few hours before spontaneous recovery, and further administration will give a new depression. Administration of a small amount of serotonin will raise body temperature. Extremely small quantities of these substances will act to produce finite effects if injected close to the temperature controlling centres.

Chlorpromazine

Dr. Domino: Are these effects strictly related to dose? The catecholamines do not have a bi-phasic effect? I gather there is only one direction of effect: small doses do nothing, large doses depress body temperature?

Dr. McIlwain: That is so, yes. However, we are interested that chlorpromazine was not developed by examining its relation to catecholamines and serotonin; the clinical effect which eventually found use was not that of lowering body temperature, but the properties which gave the description of a tranquillizing agent.

Chlorpromazine shows a quite surprising localization of a large proportion of an administered dose in the region of the basal ganglia, while the cortical concentrations are only approximately 3-5% of those in parts of the brain in which its action occurs (Tadros and Wahab, 1962; de Jaramillo and Guth, 1963). Prochlorperazine, which is also a tranquillizing agent, has a very similar distribution. As I mentioned earlier, the effective phenothiazine tranquillizers have a very different regional distribution in the brain from that of the predominantly antinauseant phenothiazines. These drugs were not selected on the basis of their localizations, but clearly they are marked by a very different localization. Remembering that the tranquillizers have been selected by their effects on overall animal behaviour or by development measures, I think that the data give quite an impressive demonstration of the way in which such guiding of drug synthesis can actually produce a drug with quite marked specificity in site of action, which can be revealed by a straightforward analysis of corresponding regions of the brain.

Dr. Brazier: Are you referring to measurements in isolated tissue or *in vivo*?

Dr. McIlwain: This is *in vivo,* with the drug given systemically. At various times from 15 to 90 minutes after administration of the drug, the animals have been killed and the brain dissected, and the different portions analyzed for their content of phenothiazines.

Barbiturates

Dr. Himwich: Dr. McIlwain, are there comparable studies for barbiturates? Many years ago, Keeser (1937), claimed that barbiturates are concentrated in the hypothalamus. But several investigators (Koppanyi *et al.,* 1934; Vogt, 1935; Domek *et al.,* 1960) have shown that barbiturates are distributed equally all over the brain. Could it be that the difference between barbiturates and chlorpromazine is not caused by differences in basic actions, but rather in distribution?

Dr. McIlwain: That is very true; barbiturates are much more uniformly distributed.

Dr. Brazier: Judging by what?

Dr. McIlwain: Again, chemical analysis of cerebral preparations.

Dr. Domino: If we were to ask you to summarize the current feeling concerning the biochemical mechanism of action of barbiturates, what would your answer be?

Dr. McIlwain: I do not think the uncoupling of oxidative phosphorylation has anything to do with the most characteristic action of barbiturates. It may play some part in the action of *some* barbiturates; there are differences between the thiobarbiturates and other barbiturates from this point of view. The thiobarbiturates do definitely uncouple, but the fact that as drugs they are more similar to other barbiturates than they are to dinitrophenol means, I think, that uncoupling of oxidative phosphorylation does not play a major part in their action, except in toxic doses.

Dr. Himwich: Would they act by a synaptic effect?

Dr. McIlwain: I think a differentiation between a synaptic and an axonal effect depends on electrophysiological rather than biochemical observation.

Dr. Brazier: May I take the opportunity to ask Dr. McIlwain whether or not he thinks that some research that I am planning is worthwhile? Dr. David Maxwell and I are considering looking for tritium-tagged barbiturate with the electron microscope to see whether we find it on the membrane of the neuron, in the mitochondria, in the glia, in the synapse, or wherever. Do you think we are wasting our time?

Dr. McIlwain: No, I think this will be very valuable information; it will not necessarily answer the question that we have been discussing, but it will give information that we will all have to take into account when we appraise the different actions.

Significance of Drug Distribution

Dr. Brazier: It seems to me that if we found it on the membranes, that would be an extremely interesting piece of information. But the question in my mind is, if you find the drug concentrated in a specific location, is that where it works? Or is it that if you find the drug at a given site, that is where it does *not* work or it would not still be there, but would have been used up? In other words when I find a drug in a certain place, is that where it is acting or where it is not acting?

Dr. McIlwain: The degree of logic in this is variable. When one finds a correlation such as I illustrated with phenothiazines, and notices that it fits in with aspects of their action, then one is happy to accept this as being a part of the action. On the other hand, when agents such as general depressants, or barbiturates, do not show such selective localization, that is because other of their properties must be conditioning the site of action. However, I think it is true that phenothiazines as a whole are more selective in their action than barbiturates. This is one of the differences between a sedative and a tranquillizing agent. The greater specificity found in the localization of phenothiazines, I think, has this significance.

Dr. Quastel: I think the interesting thing is where you do *not* find the drug, because where you do not find it, it is not likely to be acting.

Dr. Dews: But Marthe Vogt has pointed out that if you give 0.5 mg of scopolamine to a whole human being, it surely acts and changes his behaviour; yet if you look in the brain, you cannot find it because the concentration is too low.

Dr. Domino: You should look longer, and with more sensitive methods!

Dr. Dews: One could always ask, of course, will your method tell whether there are *not* molecules on the membrane? If you have a method that will detect 100 molecules or more, and your test is negative, maybe only ten molecules are needed for the pharmacological effect; and if your method is improved so that it can now detect as few as ten molecules and you still find none, maybe only one is needed for the effect. You see, it is very difficult to prove that something is *not* there in significant amount when you do not know how little you need in order for it to do something; and there are very few drugs, if any, that could be detected on a cell if only 100 molecules were present.

Dr. Kalant: You can carry this argument in the opposite direction to show that where the drug *is* may also be of no help. For example, it has been known for a long time that ethanol is distributed throughout all body water, yet obviously it produces intoxication in the central nervous system, and not throughout the body water, in muscle, bone, skin and so on.

Dr. Domino: It may be producing intoxication in muscle, but the brain shows evidence of its disturbance the most.

Dr. Kalant: Yes, but we are getting back to quantities again. Rang (1960) showed that ethanol can produce impairment of function in all sorts of tissues, but at concentrations which are very different from those that produce *in vivo* intoxication, or intoxication in the most sensitive tissues. Knowledge of distribution alone is of no use without some knowledge of concentration thresholds for effect in specific sites.

Dr. Titus: It has been the experience of our laboratory with most drugs that the activities correlate very well with plasma levels, and that distribution into one or another tissue really does not make a great deal of difference. This would certainly be the situation when the interaction between the drug and the receptor site is considered as an equilibrium reaction. If, on the other hand, you envision some of the drug effects as semi-permanent changes in the structure of the membrane, then it becomes a very different matter to interpret the significance of drug localization.

Dr. Brazier: Yes. In the figures that you have given us, Dr. McIlwain, concerning the amount of the drug in the brain, was this always the unchanged drug or was it ever its metabolite?

Dr. McIlwain: In the cases I quoted with the phenothiazines, they were almost entirely the unchanged drug. The different parts of the brain were extracted, and the extracts were examined chromatographically to show whether the drug was unchanged or metabolized. This procedure showed that it was practically all the unchanged drug, although there was some metabolism.

Storage Sites

Dr. Himwich: Dr. McIlwain, is there any possibility that drugs are slowly released from storage sites, and that their first or most potent action would appear where they were stored?

Dr. McIlwain: I do not think we are dealing with stored compounds or long term effects in these experiments.

Dr. Titus: This could be possible. Groups of schizophrenic patients who have received chlorpromazine for some time, and are then removed from the drug, will continue to show typical chlorpromazine effects for some time, so that several weeks are required for most of them to relapse (Caffey *et al.*, 1964). The inference here might be that the drug is stored in lipid and slowly released. The high lipid solubility of these drugs would be consistent with that.

Dr. Kalant: Are you referring to the lipids in adipose tissue, or brain lipid?

Dr. Titus: I believe most of the measurements were made in the lipids of liver microsomes.

Dr. Quastel: When experiments are carried out *in vitro* with chlorpromazine using isolated brain tissue, the tissue can be washed and yet the washed tissue still shows drug effects (Lindan *et al.*, 1957). Can it be washed sufficiently to restore its activity to pre-drug levels.

Dr. Titus: I think so, but I do not know how long it takes.

Dr. Quastel: Well, it is not one or two washings.

Dr. Titus: No; and I do not think that the experiment has really been done, to see how long this needs.

Dr. Himwich: You can see a prolonged action of chlorpromazine for some time after its administration has ceased. Surely the drug must be released slowly from some storage site during that period.

Dr. Titus: I suspect so.

Dr. Russell: May I ask what was the criterion measure for continued action in this case?

Dr. Titus: These were psychiatric interviews by the hospital staff.

Dr. Russell: If it is a behavioural criterion, then there are some obvious alternative explanations.

Dr. Domino: I believe you are thinking of the work of Forrest and Forrest (1963) with the phenothiazines, correlating the excretory products in the urine with clinical manifestations of antipsychotic effect.

Dr. Titus: The work I am thinking of was by Caffey *et al.* (1963). Urinary levels of phenothiazines fell rapidly after discontinuation of the drug, but appreciable levels were excreted for some time.

Dr. Domino: Forrest and Forrest concluded that phenothiazine metabolites were excreted over a matter of many weeks, perhaps months, and there might be a correlation with clinical effectiveness during that time.

Dr. Titus: Is there any possibility that, where the drug is more effectively removed into storage compartments of the tissue, it is being taken away from the site of action more rapidly?

Dr. McIlwain: Well, this would certainly happen if one were considering the body as a whole and analyzing the liver and kidneys as well as the brain; but I am not aware of it being involved within the brain itself. This is, if you like, an empirical observation made from too few examples, but I am not aware of an instance in which there was actual seclusion or occlusion of a drug within the brain itself.

Lipid Uptake of Drug

Dr. Titus: One situation I was thinking of was encountered in some unpublished experiments by Dr. J. R. Gillette, when he was measuring the inhibitory effect of phenothiazines on microsomal enzyme systems. There is enough lipid in the microsomes that they take up large quantities of the phenothiazines. The result is that if you look at the effect of phenothiazine concentration on a particular microsomal enzyme reaction, and use in your calculations the concentrations of phenothiazines originally added to the system, you conclude erroneously that there is more than one reaction involved, and that the Km values are different. In fact, that is not the case. It is just that the lipid which is associated with the enzyme-containing particles takes up large amounts of phenothiazine. If you look at equilibrium values of drug in true solution in this enzyme preparation, then it turns out that you have only one Km value, that you have only one enzyme system. But you are being confused by the lipid which is associated with the enzyme preparation itself.

Dr. McIlwain: I think that is much less likely in the case of the whole brain than in the isolated system to which you refer; and we referred to *in vivo* distribution.

Dr. Domino: There is a substance called strandin, which is present in the cerebral cortex, and to which chlorpromazine binds very avidly. Perhaps one of the reasons why chlorpromazine does not have much cortical action is that it is simply tied up as an inactive complex with strandin.

Strandin and Gangliosides

Dr. McIlwain: Yes. Strandin is an earlier name for a crude preparation of a group of about five components of the cerebral gangliosides. The substances are distributed in favour of the gray matter. There is, however, quite a considerable amount in white matter. In gray matter we usually find them present not only in cortex but also in other parts of the brain. I think the interactions that have been observed between chlorpromazine and ganglioside preparations are much less specific than these observations on distribution *in vivo.* Quite a number of other compounds will form this type of association complex with gangliosides apart from chlorpromazine. It does not differentiate between phenothiazines; for instance, methylene blue also combines with these compounds.

Dr. Hoff: With regard to the specificity and site of action of these drugs, would you agree that alcohol could be thought of as a more diffusely acting drug in general?

Dr. McIlwain: Yes, possibly even more so than the barbiturates.

Dr. Domino: This would fit in with its distribution being diffuse as well.

Dr. Hoff: May I ask a question about chlordiazepoxide? Would you list it as a more specifically acting drug?

Chlordiazepoxide

Dr. Domino: I think I would. In small doses, chlordiazepoxide will diminish afterdischarge in the limbic system, without affecting EEG activation or the knee jerk.

Dr. Himwich: Another example – under the influence of diazepam (Valium), interhippocampal responses to stimulation of the amygdala are usually facilitated (Morillo, 1962).

Dr. Hoff: Some unpublished work that we have done shows that autonomic responses evoked by stimulation of the limbic area were more readily abolished by chlordiazepoxide than those evoked by stimulation further down.

Dr. Domino: You concluded in your paper, Dr. Himwich, that this represented a stimulant action of chlordiazepoxide on the limbic system (Himwich *et al.,* 1962).

Dr. Himwich: The action is mixed.

Dr. Domino: I wasn't certain of your view. In my table I left it with a question mark (see p.61).

Dr. Himwich: Though interhippocampal responses are facilitated by the drug, the response of the hippocampus to stimulation of the ipsilateral amygdala is diminished.

Dr. Domino: We get the same effect with ethyl alcohol.

Species Differences in CNS Responses

Dr. Brazier: I think that here again one must be careful about species differences. I have recently had several opportunities to record from indwelling electrodes in the limbic system of man, and I have yet to find a single person in whom I could get a contralateral hippocampal response or a contralateral amygdala response, whereas, of course, transhemispheric responses are easy to find. So I think we must be a little careful here about species. Of course, you can turn round and say to me, well, your specimens of homo sapiens are not normal. None of them is normal – they are all temporal lobe epileptics, but I doubt very much whether this is the reason for not getting a proper crossed hippocampal response. The much more likely reason is that the dorsal hippocampus is vestigial in man, and that he has no structure closely analogous to the dorsal hippocampus of the cat.

Dr. Hoff: Dr. Brazier, did you give any drugs to these patients?

Dr. Brazier: Yes, and with very surprising results (Brazier, 1966).

Haloperidol vs. Chlorpromazine

Dr. McIlwain: Perhaps at this point we can turn to another tranquillizing agent, haloperidol. By devising a suitable spectrum of reactions to a number of tests, Janssen was able to select butyrophenones which did not display any analgesic property, but which revealed central actions like those of chlorpromazine (Janssen *et al.*, 1959; Janssen, 1964).

We obtained from him some of the animals in which he had selected haloperidol, in order to see what biochemical effects we could observe. Our data show an action on cerebral excitability measured in isolated cerebral tissues. In this property, we found haloperidol similar to chlorpromazine, but similar also to some of the compounds from which he selected haloperidol; in other words, this is a property common to several of the agents and is not specific to the tranquillizing properties in haloperidol. The respiratory rate of isolated brain tissue without stimulation is unaffected by all concentrations of the compounds examined. When the tissues were electrically stimulated, so as to produce a metabolic response, then one produces a much more sensitive system which is affected by each of these compounds without any great differentiation.

Biochemical Effects on Isolated Tissue

Dr. Dews: I noticed a very interesting cross-over effect in your data, whereby in the presence of several concentrations of, say, 'nitrochloridone' you got less respiration in the electrically stimulated tissue than you did in the unstimulated.

Dr. McIlwain: That is so, yes.

Dr. Dews: And the same with haloperidol. . . . . There is a reversal whereby electrical stimulation in the presence of certain concentrations of the drug reduces respiration. We get the same sort of thing in the behavioural effects. You start out with one form of behaviour that occurs with much greater frequency than another, and under certain doses of a drug you can find the form that would normally occur less frequently now occurring more frequently, and the normally more frequent form now occurring less frequently.

Dr. McIlwain: I admit I paid less attention to that because it is found with a high concentration of the added agent, you need about half millimolar in order to see this cross-over.

Dr. Dews: It still might help to interpret the electrically unstimulated respiration.

Dr. Brazier: Dr. McIlwain, how do you apply the electrical stimulus in your *in vitro* studies?

Dr. McIlwain: It is applied to a section of tissue, by having this under what is regarded as biochemically normal metabolic conditions, floating in a glucose-saline medium containing buffer, oxygen, and so forth. The tissue is placed between electrodes to which brief electrical pulses are applied. The essential characteristic of the stimulus is that one induces repeatedly at the tissue a momentary potential gradient of about one volt per millimetre; and it is this stimulus which results in increased respiration. It also alters the tissue concentrations of inorganic phosphate and adenosine di- and triphosphates. In measuring respiration in these systems, one is measuring an end response to a stimulus, and it is this response that is inhibited by the drugs. Chlorpromazine is inhibitory in quite low concentration, in micromolar amounts; and in the case of chlorpromazine, the corresponding concentrations of drug are found in the brain itself during its action *in vivo* as a tranquillizing agent.

Dr. Domino: Does it matter what kind of electrodes you use? For instance is it related to the metal?

Dr. McIlwain: No, in effect it is not. We usually use silver- or gold-plated platinum, but not platinum itself because it is a catalyst for oxygen uptake and induces artefacts.

Dr. Brazier: Does the tissue look the same under a microscope before and after it has had this treatment?

Dr. McIlwain: Yes, as far as we can tell. We are in the process of getting electron microscope observations. By ordinary microscope, there is no evident difference. And also the changes which are induced in chemical characteristics are reversible. For instance, on stimulation one can cause a lowering to one-quarter of the content of creatine phosphate, which is an essential constituent. This recovers, and one can follow the recovery phase also. One can also follow the loss, for instance, of potassium salts, and see how many micromoles are lost per stimulus. The quantities are in the same order of magnitude as are found in simpler neural systems, such as the squid axon.

Dr. Brazier: What makes the change in polarity?

Dr. McIlwain: These pulses are of alternating polarity, to avoid the phenomenon of polarization in the electrodes; that is, as the voltage gradient runs from one side to another of the tissue with one pulse, the next pulse a few milliseconds later has a reverse polarity.

Dr. Domino: Many years ago, I took a bi-polar electrode and placed it in saline. The parameters of stimulation were similar to those that are used to stimulate the reticular core to get EEG activation. Looking under the microscope at the electrode tip during stimulation, I saw bubbles. Presumably both hydrogen and oxygen are released by this type of current. My question is, if you damage or destroy your tissue, do you see any evidence of a change in oxygen consumption? I wonder whether the phenomenon you are observing may be related to electrolytic dissociation of water into its two parent gases.

Dr. McIlwain: No, it very definitely is not, because we are actually making some of these measurements in terms of gas pressures.

Dr. Domino: There is no change in pressure without the tissue?

Dr. McIlwain: No. And the fact that minute amounts of chlorpromazine can abolish these changes is indicative of their being metabolically produced.

Dr. Domino: You mean you have to have intact membranes for these phenomena to occur?

Dr. McIlwain: Yes. If you maltreat the tissues during preparation, or smear them, then you lose these effects.

Dr. Hoff: How thick are your slices?

Dr. McIlwain: About 0.3 mm. This is conditioned by the distances through which adequate diffusion of oxygen and metabolites can occur in such a system.

Electrical Activity in Isolated Tissues

Dr. Domino: Is it correct that you and Dr. Gerard were unable to find any electrophysiological evidence of neuronal activity?

Dr. McIlwain: Since then we have made a number of electrical measurements on the tissue itself and we find resting membrane potentials; there are also spike potentials, and the electrical stimuli have the same metabolic consequences in these preparations as neuronal activity does *in vivo.* There is also a fall in membrane polarization during stimulation; it can go practically to zero with maximal stimulation, and there is recovery when the stimuli cease.

Dr. Domino: Since your slices are in the order of 0.3 to 0.4 mm., you can make multiple slices through the cortex. Does it make any difference whether you take the slice through the dendrites as opposed to a slice through the intermediate cortex, or a slice through the pyramidal cell layers?

Dr. McIlwain: Yes. It conditions very much the number of cellular entities we can find. That is, the resting membrane potentials are found in defined areas that we can measure by the movement of the tip of the microelectrode and this corresponds to the size of a cell body. The usual distance of travel of an electrode within a negative region representing an arbitrary section across a cell is about 18 microns, rather smaller than the average cell diameter.

Dr. Brazier: Does it actually get into the cell?

Dr. McIlwain: Yes, on occasions we can go into a cell repeatedly.

Dr. Dews: Do you get any spike potential?

Dr. McIlwain: Yes, there are injury spikes often, on first entry.

Dr. Dews: But not directly caused by the electrodes?

Dr. McIlwain: No.*

Dr. Brazier: Of what are the microelectrodes made?

Dr. McIlwain: Glass micropipettes with strong KCl electrolyte solution.

Dr. Domino: This must mean that your phenomena have to be related to EPSP's** and IPSP's**, but not discharge of neurons?

Dr. McIlwain: To the membrane polarization. To continue the discussion of haloperidol, if you go to higher concentrations, it is possible to see some phenomena in which haloperidol resembles chlorpromazine. However, as these concentrations are relatively high, I don't greatly emphasize these findings in relation to the mode of action of the drug.

Dr. Himwich: Do you find that haloperidol does the same thing as chlorpromazine, but in lower doses? If you give an effective dose of chlorpromazine to a rabbit, cortical EEG alerting is blocked. With haloperidol the same effect occurs at a lower dose. So that in that regard, it seems that it is more potent.

Dr. McIlwain: No, it is not; but there have not been comparable observations, as far as I know, of the active concentrations of haloperidol reached in different areas of the brain.

General Depressants, including Ethanol

In relation to ethanol, Dr. Wallgren has applied some of the techniques that I have described to the study of its effects, and he will discuss his findings. To comment on ethanol in relation to the theme that there is a great deal in the action of all drugs which is related to patterns in which they were first selected: the selection of ethanol goes back to the time when food and drink were not clearly differentiated from drugs, and ethanol still has actions within both these categories. I often wonder how much comparative biology ought to be involved in this, knowing that human society and lower organisms have all had periods when they have been quite heavily exposed to ethanol; and whether we are derived from some earlier organisms

* Evoked potentials have since been recorded in isolated portions of mammalian cerebral tissues (Yamamoto and McIlwain, 1966).

**Excitatory Post-synaptic Potentials and Inhibitory Post-synaptic Potentials.

which were ethanol-resistant. Ethanol is a normal bodily constituent in minute amounts, and we appear to have a remarkable ability to obtain a large part of our caloric intake from it.

The biochemical actions of the general depressants as a group, including barbiturates and certain anesthetics and ethanol, are notably similar although chemically they are extremely heterogeneous substances. The likelihood is that their action does not depend much on specific individual chemical groupings, or the distance apart of charged polar parts of the molecule, in the way in which one thinks, for instance, that the actions of chlorpromazine and haloperidol may depend. Thus one is led to the fact that there is a large gap at the centre of the subject of neurochemistry that prevents us giving a logical sequence in the action of most drugs. We are happy to see the sort of correlation between localization of chlorpromazine and its action, or correlations such as those between what are in effect different aspects of the action of chlorpromazine and haloperidol. What we would really like is fundamental knowledge of how the neural impulses are initiated and spread in any system, not only in the CNS but in other neural systems. In chemical terms this means how does the permeability of the nerve membrane alter so that one obtains first of all the sodium entry and then the potassium outflow which make the electrical phenomena of the nerve impulse? Until we understand these we won't have a chemical logic to the action of substances even as simple as ethanol.

General discussion

DR. KALANT, *Chairman*

Dr. Kalant: Without intending in any way to limit the discussion, I would like to suggest that it might be profitable to raise at the beginning a few main problems or areas of discussion, which can serve to orient the conversation. I have jotted down the following points for your consideration:

Areas of Discussion

The first is the question of localization of drug effects within the nervous system; the matter of whether or not, on the basis of the evidence that Dr. Domino presented yesterday, it is possible to talk of sites of action and to raise some suggestions about possible sites; and whether or not these localizations of action, if they exist, are adequate to account for the observed similarities and differences in behavioural effects of the various drugs that concern us here.

The second main area which I should like to suggest is the question of biochemical mechanisms which have been explored to date. Which of these, if any, seem most likely to explain the differences in localization and in behavioural effects which we may possibly agree upon in relation to the first question? Do some biochemical mechanisms seem most appropriate to account for similarities of action, and others to account for differences of action which may be described?

The third main area is in relation to the behavioural effects. Do any of the biochemical effects which may be discussed offer a suitable basis for the postulated secondary stimulus or derived stimulus, which Dr. Russell mentioned in his scheme yesterday? Do any of these satisfactorily explain a sustained drive for drug intake in the process of addiction? Of the various things that have been mentioned, which, if any, appear to fulfill this requirement?

Would anyone care to suggest any other major area for discussion?

Dr. Russell: It seems to me that running throughout discussion and our formal papers as well, were a number of questions of methodology.

It seems very important, at this stage of development in research in this field, that various issues of methodology come out as we discuss the three main topics you have raised. I would hope, for example, that we could return to the question Dr. Domino raised yesterday, about definition of minimal effective dose. Is there some systematic way of applying this definition statistically, that will satisfy everyone and that can be used in an objective way? There are also serious problems in selecting the behavioural measure to be used. Any drug may cause a variety of changes in behaviour, and one's choice of measure of behaviour may determine whether one finds enhancement, impairment, or no effect.

Dr. Himwich: Also, what dosage?

Dr. Russell: Dosage relationships are indeed essential points to consider.

Dr. Kalant: In the course of our discussion, we shall likely agree on some problems which we consider most worthy of high-priority investigation, and probably questions of method will be very high on our list.

Dr. McIlwain: In any proceedings or recommendations, there should be some comment on the origins of the drugs, and consideration of the processes of their selection. To simply accept drugs without taking into account their origin will give great artificiality. I think that there should also be a strong recommendation for including testing against addictive properties, at a very early stage in the production of new drugs.

Dr. Dews: I think there is a corollary to this. When one comes to examine the evidence for the behavioural effects of drugs a great many things that we take for granted are not as well established as we think. A great deal of work remains to be done simply on what the drugs do to behaviour. A lot of specificity has been assumed on very little basis.

Dr. McIlwain: In other words, what is the analogue to, say, the Straub sign, which might correlate with addictive properties in other drugs?

Dr. Kalant: In the case of the opiates, this problem has been solved recently by the test of ability of a new drug to maintain an addiction already established (W.H.O., 1964b). The question, perhaps, is whether the risk of addiction to other types of drugs can be similarly predicted by deriving a good experimental model which would permit early testing of the drug.

Dr. Dews: I think Dr. McIlwain has a good point; the very nature, the laboriousness of the sorts of testing procedure for addictive properties that are now being used, means that you can only go through a few drugs a year. Therefore a drug probably gets to be tested at Lexington or Michigan, with respect to its addictive propensities, only after it has gone through all the rest of the preparations for clinical use. Some easier method of prediction is needed, that could be applied to compounds by the hundreds at a very much earlier stage in their evolution, so that the evolution of new drugs could be directed away from addiction propensity. And if I understand your thesis, in order to use this evolution effectively, so to speak, you must have a large population of drugs prepared so that you have the selective emergence of new strains or species of drugs. Is this not so?

Dr. McIlwain: Yes, this is an identical view. I mentioned this specifically in case we are to consider what recommendations should be made.

Dr. Kalant: Perhaps we should include as a fourth area, for discussion and suggestions, the question of what characteristics of action, and what structure-activity correlations, may give rise to means of testing for addictive properties. If these questions are agreeable to everyone, I would propose that we open the discussion by asking Dr. Domino to restate very briefly his views on the validity of present concepts of localization of drug action.

A. PROBLEMS IN STRUCTURAL LOCALIZATION OF DRUG ACTION

Neuronal versus Area Levels in CNS

Dr. Domino: I think it is necessary for us to consider what is going on at the neuronal rather than at the area level in the brain. It is probably insufficient to talk about drugs that act on the cortex, thalamus, etc. Frequently, current electrophysiological techniques cannot tell us accurately what is going on; and furthermore, what may be going on is a summation of both excitatory and inhibitory events at a given level of the brain. Moreover, different synapses in the same anatomical area may respond quite differently. Augmenting responses, which are in general mediated over cortical axosomatic synapses, may be very markedly depressed by a given dose of ethyl alcohol, while recruiting responses, mediated over axodendritic synapses, are not depressed as much. One has to be very careful in interpreting which part of the evoked potential is studied. If one is to pursue this investigation from a biochemical point of view, one ought to isolate these different synapses or cell systems and study how they are affected by ethyl alcohol and the other drugs in question.

Dr. Quastel: When we are dealing with brain, we have to deal with a total of about seven thousand million brain cells. I cannot bring myself to believe that when a drug enters the circulation, it is going to affect seven thousand million cells all in the same way. I would be very surprised if more than a very small proportion of those cells which take up the drug are affected. Those that are affected will be the ones which presumably have the highest 'affinity', or those which are most susceptible at the concentration of drug which is present. As we increase the concentration of the drug, more cells will be affected. Now the chemical composition of the brain is not uniform and I expect that the phospholipids and other lipid components of membrane are also going to vary both qualitatively and quantitatively in various parts of the brain. It stands to reason, then, that if we have two substances of different chemical constitution, they will affect different parts of the brain even if the mode of action of these drugs on the neurons is identical, biochemically speaking. The clinical effects will vary according to the sites in the brain that are affected. So when we talk about localization of action of a drug, what do we really mean? It may represent the parts of the nervous system for which the drug has the highest affinity, or those places where it exerts the largest effects on cell function. The very sites in the nervous system affected by the drug will vary according to the dosage, and the clinical responses will differ according to the parts of the brain that are affected.

Susceptibility to Drug versus Vulnerability of Function

Dr. Kalant: Would you feel that many of the phenomena which are studied biochemically in, let us say, isolated portions of cerebral cortex should be repeated with other portions of the brain to see if there is a spectrum of different susceptibilities of the same process to the same drug in different areas?

Dr. McIlwain: That is certainly my view.

Dr. Dews: When you say 'parts of the brain', Dr. Quastel, what sort of subdivisions have you in mind? Is your thesis that there are different types of neurons doing the same work but in different parts of the central nervous system?

Dr. Quastel: Chemically speaking. . . . .

Dr. Dews: Recognized by their susceptibility to different agents?

Dr. Quastel: But each may be performing physiologically the same job.

Dr. McIlwain: There is also scope, as has been indicated, for differences in the relative effects of different drugs in different parts of the nervous system. What, then, can we say biochemically about localization of the action of a drug? I think there are two main categories to this. One is, that in some instances a drug is differentially localized even if its action is uniform; the phenothiazines are examples of this. In the other situation, in which there can be a uniform concentration throughout, there may be a different balance in the actions of a drug in different parts of the nervous system. Now this can be conditioned, I think, not only by differences in chemical makeup but also by differences in anatomical makeup. One pictures that much of the membrane of a neuron is fairly similar; nevertheless, where there is great ramification of dendrites, there is a different relation between membrane properties and cytoplasmic properties. Even if there is not a different fundamental action of a drug this anatomical characteristic can cause a difference in its action in different parts of the same neuron. Actually, of course, it is fairly certain that there will be differences in chemical makeup of the neuronal membrane at different points where it has different functional characteristics, such as at synaptic regions, or at other axonal regions.

Specific Areas versus Specific Pathways

Dr. Dews: Is there any reason to suppose that those cells that are particularly receptive to, say, morphine will be aggregated in a particular chunk of the central nervous system? Isn't it much more likely that there are different systems of neurons with different chemical characteristics that are more or less widely distributed throughout the brain – perhaps more concentrated in some places than others, but nevertheless quite widely distributed? When we talk about drugs affecting different parts of the brain, perhaps what we really should be talking about is drugs affecting different systems in the brain, although the cells of a particular system might have higher concentrations in some places than others.

Dr. Quastel: Could be.

Dr. Dews: And this means that you have got to go about studying the localization of action in terms of a very fine pattern of organization, rather than looking for the effect of a drug on '*the* thalamus' or on '*the* cortex', or on '*the* reticular formation.' *

Dr. Hoff: In that connection it may be that if you study the system, you will find that certain neurons that originate a systematic response are the more vulnerable ones, and a drug which affects them may, therefore, secondarily affect others which are not necessarily more vulnerable *per se* to the drug.

Dr. Himwich: May it not be that some parts of the brain have more of one kind of cell than another?

Cellular Distribution in CNS

Dr. Dews: Right. The cells of a particular chemical system need not be uniformly distributed. On the other hand, they are unlikely to be sorted out in such a way that a particular chunk of brain is composed exclusively of cells of a single type. The situation will probably be somewhere in between, with cells of a particular morphology being widely but very unevenly distributed.

Dr. Kalant: If your concept is that there are functional systems distributed more or less in parallel throughout all levels of the nervous system, but perhaps differing to some extent quantitatively, the question of localization of a specific function would depend on how great the quantitative differences in distribution are. Can we agree on any examples that would suggest that there are, at least for some of these cell systems, sufficiently different localizations to make anatomical localization studies worthwhile? For example, is the cortical activating function of the ascending reticular system not sufficiently well localized to imply that some kind of cell is present in a substantially larger concentration there than in other parts of the nervous system?

Dr. Dews: I would like to give an example that came up yesterday. If you look at the effects of morphine on the reticular formation, you find that within a few millimeters, in a rat's brain at least, morphine has the effect of inhibiting respiratory function and, at the same time, an effect in enhancing vomiting.

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* Ed. Note: As an example, see Towe (1965).

Dr. Kalant: But a few millimeters, in relation to cell sizes, constitute a large and physically separable distance. Neurophysiological techniques are now sufficiently good that one can distinguish differences in electrical activity and in response to stimulation, of points a few millimeters apart.

Dr. Dews: I just don't know the answer. Are the cells mediating these functions separated, or are their processes interlaced, with some of the pathways used in common? Respiratory-type movements are certainly used in vomiting.

Dr. Domino: I suppose it depends on what neuroanatomist you are talking to as to how separated or how diffuse they are.

Dr. Himwich: I can give another example. In most mammals, if you extirpate the amygdala and the anterior half of the hippocampus, you get quite definite, serious behavioural changes: there is more orally-directed behaviour, hypersexuality, especially in males, and a loss of learning from previous experience. If you take out the septal area of most mammals, especially of rats, they become hyperirritable, so that a stimulus that would ordinarily not bother them, will now make them turn with rage at the source of stimulation. This is something for which it is very difficult to pinpoint specific neurons. Maybe later we shall be able to do so, but right now it is more convenient to think of areas, at least in explaining behaviour.

Dr. Brazier: You are speaking of bilateral removal of the amygdala — bilateral removal of the anterior hippocampus?

Dr. Himwich: In the experiments of Klüver and Bucy (1939), the removal of the temporal lobes was bilateral.

Dr. Brazier: In man a unilateral amygdalar lesion will not induce any of these changes. It is done all the time for treatment of temporal lobe epilepsy. We don't do bilateral lesions, of course, because it ruins the memory.

Dr. Himwich: What about infra-human animals?

Dr. Hoff: There seems to be species differences in bilateral versus unilateral susceptibility. Konorski (1961), for instance, showed that unilateral ablations in dogs produced behavioural defects that others felt were only produced by bilateral pre-frontal lobotomy in primates.

Dr. Brazier: May I just say one thing before we move on? Let us not think only of millimeters. Let us think perhaps of safety factors. There may be some synapses that have no safety factor. Therefore, if you impair their input, they are thrown out of the picture altogether; they are blocked. There may be others which will fire with only a minimal number of their own inputs being active at the same time, so that you can take out 30% of their inputs and they will still fire. This might be the difference in susceptibility that Dr. Quastel was talking about, between some cells and others.

Synaptic Safety Factors

Dr. Hoff: That is an extremely good point.

Dr. Kalant: I wonder if the contribution of biochemical studies to localization of drug action will have to be on the basis of biochemical differentiation of cell types or functional systems, rather than in terms of single biochemical processes that are affected diffusely throughout the nervous system by drug action. Dr. McIlwain mentioned earlier the possibility that a drug may act upon the same process at all levels of the nervous system, but more so in one part of the brain, or in one functional system, than in another. Another possibility might be that the process which is affected by the drug may be more closely related to the function of one part of the nervous system than of other parts. Or, as Dr. Brazier suggested, another basis of localization might be in terms of a safety factor; a process which is affected by a drug diffusely may have less reserve capacity in one part of the brain than in another under normal operating conditions, and therefore be more readily suppressed by the drug at lowest concentration.

Summary

If this is so, one would have to conclude that measuring the effect of the drug upon some particular isolated biochemical process *in vitro* can only give leads with respect to which biochemical processes to study in different areas of the brain, rather than provide an explanation in its own right. Would this be a reasonable assessment of the situation so far?

Dr. McIlwain: I think so. Necessarily one is operating at a simpler level in doing what one is doing biochemically. If one is operating in whole animals, then one is in effect performing physiological and pharmacological experiments as well as a biochemical one; but that type of experiment gives the initial lead as to what part of the nervous system one should pay attention to. If one, in looking biochemically at several parts of the brain, does find a correlation which directs one's attention to the same area as electrophysiological methods do, then one has a greater assurance that the biochemical effects being studied are actually related to the primary action of the drug.

B. NEUROPHYSIOLOGICAL STUDIES FOR LOCALIZATION OF DRUG ACTION

Relation of Specific Effects to Mechanism of Significant Effect

Dr. Dews: What I am a little uncomfortable about, is that the history of physiology shows that it is very hard to find the mechanism of a phenomenon if you do not know what the phenomenon is whose mechanism you are looking for! There are differences between chloralose and barbiturates, and small differences between the various barbiturates in their pharmacological effects. But these differences have been poorly characterized, especially at the behaviour level, which is the only significant level with respect to the economy of the animal as a whole, or to clinical use. If you do not know the nature of the significant effect, it is very hard to know whether some specific cellular change you have found is related to the mechanism of the significant effect or not. All these drugs have many effects at the cellular level. Which are important for the overall effects on the animal, and which are incidental?

Differences Among Barbiturates in Specific Neural Test Systems and in Addictive Properties

It is essentially impossible to determine this until we understand the nature of the behavioural effects. Dr. Domino yesterday referred several times to quite marked differences between barbiturates in specific neural test systems. So far as clinical authority is concerned, if you exclude epilepsy, the barbiturates are used very much interchangeably. Whether you prescribe secobarbital or pentobarbital depends on your present preference in prescribing, more than on anything else; there are no good behavioural tests that I know of that bring out any real distinctions between amobarbital, pentobarbital, secobarbital, and even phenobarbital. So one is faced with the question – 'Are these differences to which Dr. Domino refers of any real significance in determining the overall effects of the agents?'

Dr. Kalant: I would like to agree very heartily with what Dr. Dews has said, not only because it is true of the various barbiturates, but also because one may draw very close analogies between the effects of barbiturates and those of ethanol and other depressants. For example, an early phase of gross behavioural excitation (regardless of whether or not this is a manifestation of selective neuron depression), is also seen with very small doses of barbiturates in many subjects. And the effects, when one gets to the stage of sleep or coma, are extremely difficult or impossible to differentiate. I should like to ask whether *any* of the behavioural effects are sufficiently specific to let us say that one of these drugs is really different from another. If there are qualitative or quantitative differences which do permit differentiation, do these differences correlate with some relatively gross difference in electrophysiological effect in different functional parts of the nervous system, or in biochemical effect upon some particular cellular type?

Dr. Dews: I do not want to be misunderstood. I am not against neuropharmacological analysis on any level that is possible. All I am arguing for is that much more work is needed to analyze the overall effects on the whole animal. Many neuropharmacologists and biochemists seem to suppose that the behavioural effect of, say, pentobarbital is simple and well known ('It is a depressant!'). Nothing could be further from the truth. Also, it would be well to have greater caution in interpretation of particular neuronal effects which are found, rather than the immediate assumption that the latest effect described is going to explain all the pharmacological actions. I am not against the analysis, only against the misinterpretation.

Dr. Brazier: I would like to disagree with Dr. Dews and with Dr. Kalant if I may. I think that there is a big behavioural distinction between the barbiturates in that, in man, you cannot get abstinence symptoms from the slowly eliminated barbiturates. I do not know how we are defining addiction, but it usually includes the idea that on withdrawal you get 'withdrawal symptoms.' Well, you cannot get the usual withdrawal symptoms from barbital (veronal) or from phenobarbital (luminal). One of the withdrawal symptoms that you can get with the short acting barbiturates is that the subjects become sensitive to photic stimulation, as some epileptics are. They show a paroxysmal discharge in the EEG. You do not get that with veronal or with luminal. There is a very big difference between short acting and long acting barbiturates. Will you agree with me, Dr. Wallgren?

Dr. Wallgren: I do not really think there is a point of disagreement. I think Dr. Dews and Dr. Kalant were speaking about the effects of a single acute dose, and not of the addictive problem.

Dr. Domino: Dr. Brazier, you *can* show withdrawal symptoms to barbital in dogs. Nevertheless, the point you are making is a very important one and I agree with your philosophy. Basically the long-acting barbiturates are not clinically a problem in terms of physical dependence and withdrawal symptoms, in contrast to the intermediate or short-acting barbiturates. This is related to the fact that the long acting compounds are not euphorigenic, while the short-acting are. This crucial difference is what accounts for the fact that we have so much abuse of the shorter acting barbiturates in man.

Dr. Brazier: There is a monograph on this from Copenhagen (Wulff, 1959). It deals with man, and describes a large number of cases – 70-100 cases – of this and that drug. The investigators made EEG recordings and never found the paroxysmal discharge from barbital or phenobarbital withdrawals.

Dr. Dews: The only really carefully controlled programme that I know of, in connection with production of withdrawal symptoms by the intermediate-acting barbiturates, was the study at Lexington (Isbell *et al.*, 1950). With very large doses of one of these intermediate barbiturates, they produced very frank withdrawal symptoms. The milligram for milligram potency of phenobarbital is much less than that of pentobarbital. Addicts would have to take astronomic doses of phenobarbital for equivalence. So you have a dose difference. The second point is that it is well established, clinically at any rate, that if you give an epileptic phenobarbital, then withdraw it from him, the risk of epileptiform convulsions during the withdrawal is very much greater than the incidence before he ever had phenobarbital.

I think a study should be done in experimental animals, of whether there is a difference between phenobarbital and amobarbital with respect to withdrawal symptoms.

Dr. Brazier: I agree about that on epileptics, of course, but I am not sure about euphoria.

Dr. Kalant: Is that not largely a matter of difference in the time-course of response? Phenobarbital is fairly substantially protein-bound. Its time course of action is a slow one with a low peak and a long duration. This fact, in itself, may prevent to a considerable extent the development of euphoria as well as of withdrawal symptoms. This might be comparable to the difference between heroin and methadone.

Dr. Brazier: Phenobarbital tends to accumulate on repeated doses, so that the blood level goes up, or at least the effect of a dose has not worn off by the time the next one is given. But the effect of a short-acting one has worn off. Now has this got something to do with the fact that it is so difficult to get phenobarbital withdrawal symptoms in man?

Dr. Titus: What about simple lipid solubility? The short-acting barbiturates are much more lipid soluble than any of the long-acting ones, and this may very well be one reason why they are short-acting, because they probably reach the liver microsomal enzymes more rapidly.* We can ask, perhaps, whether the production of withdrawal symptoms depends on some distortion of the lipid structure of the neuron, quite apart from the fact that a barbiturate is causing it; the important thing then might be that it is a more lipid-soluble barbiturate.

Dr. Brazier: This is the kind of thinking that lies behind the electron micrographic localization of tagged drugs that we are beginning to do, starting with the barbiturates.

Dr. Hoff: Returning to Dr. Kalant's remarks on the possible significance of differences in the time-course of action of the barbiturates: I wonder if this might have relevance in the examination of euphorigenic properties of ethanol? How would we transfer this information over to that field?

Dr. Kalant: The question, I suppose, is: if one takes those barbiturates which *are* euphorigenic, what differences and similarities can we find between the actions of these drugs and others which are used in a similar manner, and those of ethanol? Dr. Himwich, would you care to comment?

Measure Drug Effects at Various CNS Levels

Dr. Himwich: I would like to underscore Dr. Dews' remark in regard to observations made at several levels. It would appear important to study different levels because the findings may represent different actions. For example, intravenous administration of adrenaline may evoke EEG alerting, an action mediated at the level of the midbrain reticular formation. Yet in response to this 'stimulating' effect of intravenous injection of adrenaline, 80% of the individual reticular neurons examined showed a decrease in firing rate and only 20% showed an increase. Moreover, this relationship is reversed in the posterior hypothalamus (Baust and Niemczyk, 1963).

Dr. Hoff: Yes, because an area is more than the sum of its neurons.

Dr. McIlwain: The picture which Dr. Domino has developed is certainly susceptible to experimental study. I would be very happy to

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*Ed. Note: For a more generally accepted view, see Crawford (1966).

CNS Transmitter Agents and Drug Action

see it tackled in terms, say, of acetylcholine metabolism in the different parts of the brain, in different conditions. However, the observed level of activity of an enzyme often depends on a question of access and supply of co-factors, rather than simply the concentration of enzyme. Especially when it is a question of a short-term adaptation within a matter of, say, minutes or an hour, one would tend to think of an enzyme becoming more accessible or becoming more activated rather than increasing in net amount. The turnover of enzymes in certain tissues is known, and information has been given about how soon one can expect an enzyme to increase by a given amount. Very little is known in the brain from this point of view. This is a lack in our fundamental knowledge: how quickly, say, enzymes acting on acetylcholine or ATP, or even lactic dehydrogenase, are renewed. This naturally will condition the speed at which they are likely to change in quantity. But the degree to which an enzyme *activity* is exhibited will depend very much on levels of intermediary metabolites, such as adenosine diphosphate. In fact one does see changes in the levels of simple metabolites quite promptly during the action of a number of the drugs we have been discussing. Ethanol, barbiturates and some of the phenothiazine tranquillizers do bring about quite appreciable changes even in the total brain level of inorganic phosphate and ADP, indicating metabolic adaptations at the level of metabolite concentration.

Dr. Brazier: What is the current opinion of informed people about the identity of transmitters in the brain?

Acetylcholine as a CNS Transmitter

Dr. McIlwain: I think one has to take acetylcholine as the substance about which most is known and which is very clearly a transmitter; and others are becoming more recognized.

Dr. Brazier: Do you feel there is conclusive evidence for acetylcholine being a transmitter in the brain? I know of no proof.

Dr. McIlwain: There is its release on stimulation, there is the fact that when acetylcholine metabolism is blocked, transmission is affected.

Dr. Brazier: In the brain?

Dr. Dews: Acetylcholine is present in the brain. It is also synthesized there. If you give physostigmine, which interferes with the hydrolysis of acetylcholine, you get changes in brain function. If you analyze saline which has been put into a shallow cylinder sitting directly on the exposed surface of the cortex, you can show that when there is activity in the underlying brain there is increased liberation of acetylcholine. When you reduce the activity, the liberation declines.* If you give substances that interfere with the action of acetylcholine, you can also get changes in brain function.*

Dr. Brazier: These facts are all circumstantial evidence, not proof.

Dr. Domino: They could all be used as arguments for a metabolic role of acetylcholine.

Dr. Dews: None of these point crucially to a transmitter action; acetylcholine could be a modulator of transmission, or something of that kind. Collectively, it seems to me that these facts show that acetylcholine is important. The fact that its liberation varies with the level of activity in the cortex, I think, comes closest to establishing at least a close correlation between interneuronal transmission and acetylcholine release. To me, it is all circumstantial evidence; but, as Thoreau is supposed to have said, circumstantial evidence can be quite convincing, as when one finds a trout in the milk.

Dr. Himwich: Some scientists accept acetylcholine as a neurotransmitter, but only in peripheral nerves and in the spinal cord. There is, however, circumstantial evidence that acetylcholine may also be a neurotransmitter in the brain. Acetylcholine concentration rises during sleep and during anesthesia; probably the rate of utilization is lower. Moreover, a group of Swedish scientists has been building up quite a body of evidence indicating that dopamine, noradrenaline and serotonin may also be neural transmitters (Falck, 1964). In making analogies for the function of acetylcholine we usually think of its rapid production and breakdown in the neuromyal junction. But it is also accepted that acetylcholine is the neurotransmitter between the vagus and the heart. Yet when acetylcholine is liberated at terminations of the vagal nerve in the heart there is a slow and gradual response that is entirely different from the rapid one occurring at the neuromyal junction.

Dr. Hoff: An additional item of evidence is that when we deafferented the cord, we could correlate the degeneration of the synapse with the reduction of acetylcholinesterase activity (Nachmansohn and Hoff, 1944).

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*Ed. Note: For further references, see Collier and Mitchell (1967), and Kanai and Szerb (1965).

Dr. Brazier: I can see that for the spinal cord, but not centrally.

Dr. Domino: Why make such a big difference between spinal cord and cerebrum?

Dr. Brazier: I would like a little more direct evidence and less analogy from peripheral axonology and spinal cord physiology.

Dr. Domino: Your point is absolutely sound, and the purist, as of today, cannot say that anywhere in the central nervous system is there a proven neural transmitter function for acetylcholine. On the other hand, I think that, as Dr. Dews has pointed out, the total of present evidence would certainly suggest or imply that it does have an important role.

Dr. Himwich: Histochemically, Krnjevic and Silver (1965) have shown that the last most rostral link in the process of EEG arousal, probably lying in the V and IV layers of the cerebral cortex, is undoubtedly cholinergic. We also have found pharmacological evidence in favour of that concept (Cuculic, *et al.,* 1967), although I admit that it is all indirect.

Acetylcholine and Permeability

Dr. Domino: One thing I would like to emphasize is the fact that the red cell membrane contains a great deal of acetylcholinesterase. Since red cells have no neuronal function, acetylcholine is presumably playing a metabolic or ion transport role. An item I saw in Chemical Abstracts reports that cholinergic agents influence the ability of influenza virus to penetrate the red blood cell. Incubation of either acetylcholine or eserine tends to prevent the influenza virus from going into the red cell. Here again is evidence to suggest that metabolic or permeability roles for the cholinergic system are quite important. Furthermore, bacteria involved in the fermentation of hay produce a considerable amount of acetylcholine. Likewise the potato is said to contain a high concentration of acetylcholine, and yet it certainly has no neurotransmitter function here. Also, bananas have high levels of serotonin and catecholamines. There must be important metabolic roles for these substances as well.

Dr. Kalant: Why do you imply that there is a difference between a metabolic role and a neuro-transmitter role?

Dr. McIlwain: I am sure it is well known that permeability changes have a different significance in the nervous system from their significance elsewhere; the common action of acetylcholine might be on permeability, and the difference lies in the biological use to which the permeability change is applied in different tissues.

Ethanol, Transmitters and Membrane Function

Dr. Wallgren: We seem to be in agreement that we do not really know much about the function of transmitter substances in the central nervous system. But dealing with the point that Dr. Domino raised in the discussion, I agree first of all that it would be very important to study variation in levels and rate of metabolic turnover of transmitter substances during the action of drugs, especially during prolonged action, the development of tolerance, and withdrawal.* Tying that up with what Dr. McIlwain just said, I think another very important point is what do these transmitter substances do? Presumably they somehow modify membrane properties. Again, we are rather vague about the implication of the term ‘membrane’, where it is located, and so on, but we have a general idea which is largely based on the work done by Hodgkin and Huxley on squid axon. In the alteration of the properties of the excitable membranes, presumably, a transmitter or moderator substance is involved. But research on the effects of ethyl alcohol has shown very little evidence of real change in the levels of various candidates for transmitter functions; it certainly has been too little studied, and I think it should be pursued much more.

We also need much more precise and extensive data on the electrophysiological level. We should consider the possibility that ethyl alcohol, for instance, does not act by altering levels or metabolism of transmitter substances, but alters some property of the membrane so that it responds in a different way to the transmitter. That is an important problem, but for different workers, using different techniques.

Dr. Domino: When you say a membrane, are you thinking in a very general sense of membranes?

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*Ed. Note: Later work (Kalant & Grose, 1967) suggested that the development of tolerance to ethanol is associated with a decrease in the effect of ethanol on acetylcholine release in cerebral cortex.

Dr. Wallgren: Not really, because although we have many membrane structures, such as the endoplasmic reticulum system and the mitochondrial membrane, the relevant structure here is the excitable membrane which has the specific property of being able to conduct impulses.

Dr. Kalant: I should like to raise a question which is of interest partly because of its educational application. Based on the old Jacksonian concept that anesthetic agents produce a progressive depression beginning in the cerebral cortex and extending downwards, it is still commonly stated that ethanol affects the highest intellectual functions first, and produces a progressive descending depression as the dose is increased. But anesthetics are now thought to act primarily not on the cortex, but on the reticular activating system. Is there sufficient evidence to warrant a decision about where ethanol acts, in comparison with anesthetics, barbiturates and other 'depressants'? Perhaps Dr. Himwich would care to comment.

Comparative Susceptibility of Various CNS Sites to Ethanol

Dr. Himwich: By way of commenting, I shall outline briefly the results of some electrophysiological studies we have done on the comparative susceptibility to ethanol of the spinal cord, thalamus, certain discrete areas of the cerebral cortex and the midbrain reticular formation in cats immobilized with d-tubocurarine. The details of the experimental design have been published elsewhere (Dravid *et al.,* 1963; Schweigerdt *et al.,* 1965; Himwich *et al.,* 1966). Suffice it to say that recording electrodes were implanted stereotaxically in the sites mentioned. The cortical electrodes were placed in the somatosensory area in the region of the cruciate fissure (S.2), as well as more medially and posteriorly near the coronal suture (S.1) (Fig.2). Two to three hours after recovery of the animals, the central stump of a cut superficial branch of either the radial or the sciatic nerve was stimulated and the evoked potentials were picked up at the recording sites under examination, both during a control period and at specific times after the intravenous injection of ethanol in a dose of 1 g/kg. This dose was found to be the lowest one to show a gross behavioural effect in the form of a mild ataxia of the hind legs.

The effects of alcohol on the response from the lateral funiculus (LF) of the spinal cord at the level of the 2nd cervical vertebra and from the contralateral reticular formation (RF), following stimulation of the radial nerve, were compared in 9 cats. The response from the lateral funiculus was composed of a double positive deflection lasting about 20-24 msec and followed by a longer and less intense negative

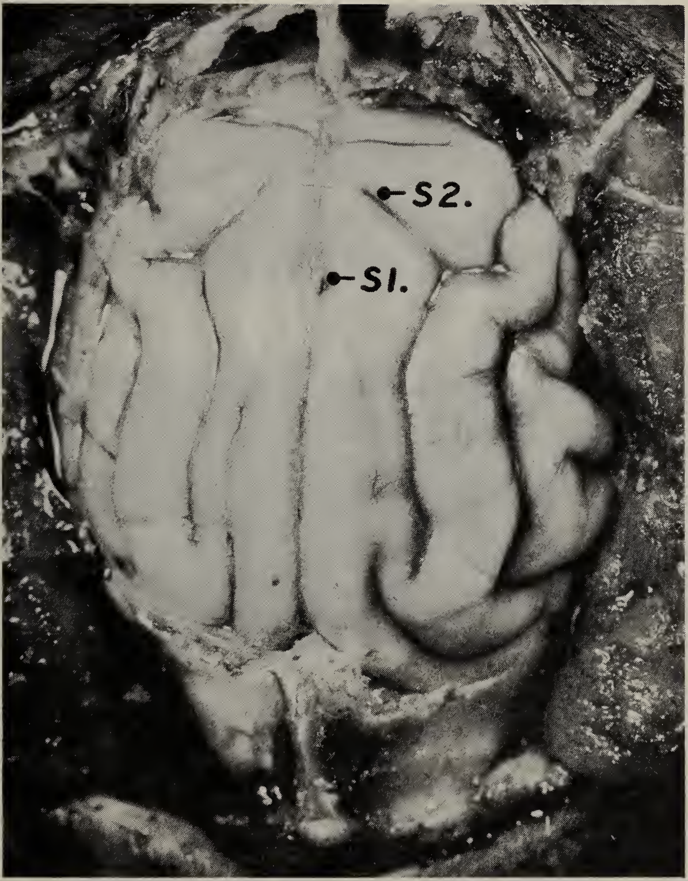

Figure 2

Two sites used for evoked potentials, one in the somatosensory cortex (S.2) in region of the cruciate fissure, and the other (S.1) somewhat posterior and medial near the coronal suture. Reprinted from Himwich *et al.* (1966), Psychosom. Med. *28* (No. 4, Part II), with the kind permission of the publishers.

wave; the first positive spike, with a latency of 2 msec, was not affected at all by the dose of alcohol given, but the second spike, with a latency of about 10 msec, was depressed 5 minutes after alcohol, as was that obtained from the reticular formation. The lateral funiculus had almost fully recovered 15 minutes after alcohol, while the reticular formation still showed partial depression at 60 minutes (Figure 3).

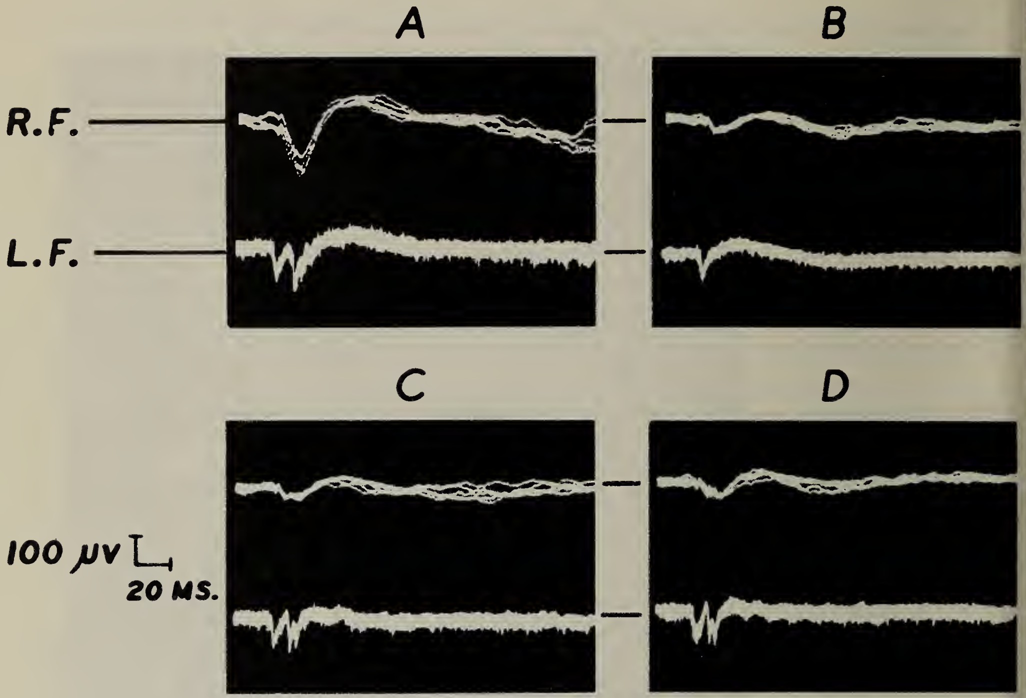

Figure 3

Evoked responses to electrical stimulation of the radial nerve recorded from the contralateral midbrain reticular formation (RF) and homolateral lateral funiculus (LF) of the spinal cord at C_2 level.

A, control; B, C, D, respectively 5, 15 and 60 minutes after intravenous injection of ethyl alcohol (1 gm/kg). A depression is evident in both responses (B), the lateral funiculus is almost fully recovered 15 minutes after alcohol administration (C) while the reticular formation is still partially depressed at 60 minutes (D). Note the first spike of the LF was not affected at all by this dosage of alcohol.

In this figure and in the following ones each trace displays the result of five superimposed frames; upward deflection indicates negativity. Reproduced from Di Perri *et al.* (1968). Quart. J. Stud. Alc. *29*: 20, with kind permission from the publisher.

When recordings were taken from the caudal end of the contralateral VPL* and from the area designated as S.1, near the coronal suture, homolateral to VPL, the evoked cortical response showed no change in latency but its amplitude was markedly decreased, and even 60 minutes later was not fully restored. The thalamic response (VPL), on the other hand, was hardly affected by this dose of alcohol (Fig.4).

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* VPL = nucleus ventralis postero-lateralis of the thalamus.

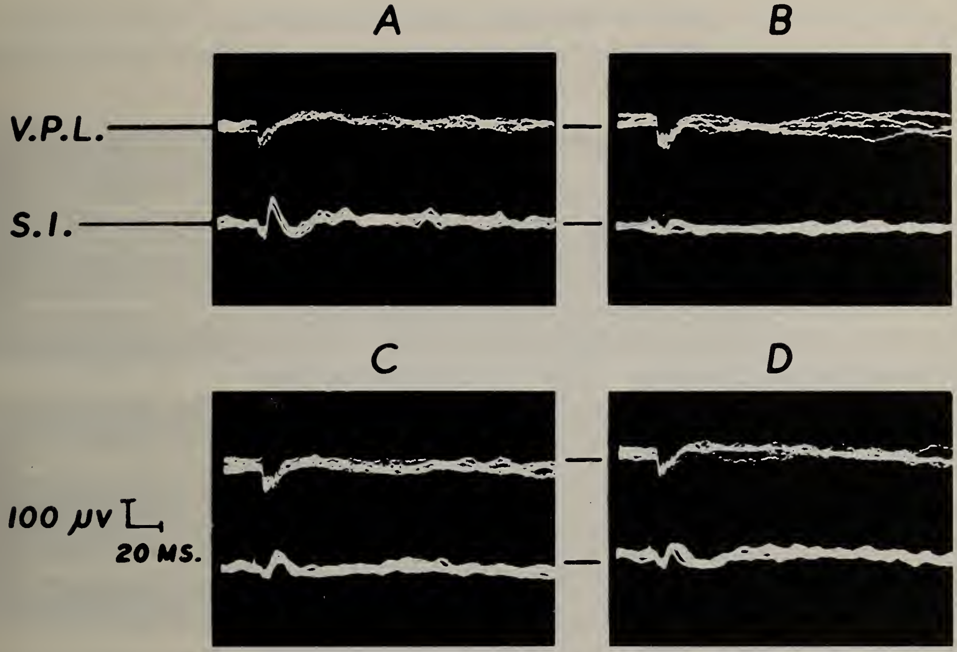

Figure 4

Evoked responses to electrical stimulation of radial nerve recorded from the contralateral nucleus ventralis posterolateralis (VPL) of the thalamus and from the area (S.1) near the coronal suture, homolateral to VPL.

A, control; B, C, D, respectively 5, 15 and 60 minutes after intravenous administration of ethyl alcohol (1 gm/kg). The response from VPL is hardly affected while marked depression of the cortical response is evident (B) and continued though to a lesser degree one hour after the injection of alcohol (D). Reproduced from the Quart. J. Stud. Alc. *29*: 20, 1968, with the kind permission of the publisher.

Dr. Kalant: If you had an inhibitory effect on the midbrain reticular formation, would you not expect it to reduce the response evoked at S.1 by peripheral stimulation?

Dr. Himwich: I would prefer to consider that point in our subsequent discussion. When we recorded simultaneously the evoked responses from the contralateral midbrain (RF) and the somatosensory area designated as S.2 in 6 cats after ethanol, we found both responses were depressed at 2 minutes, both showed some evidence of recovery at 15 minutes, but at 60 minutes the cortical response had mainly recovered, though the reticular formation still exhibited a marked degree of depression. Thus, in this series, the reticular formation exhibited a greater degree of depression by ethanol than the somatosensory cortex (Fig. 5).

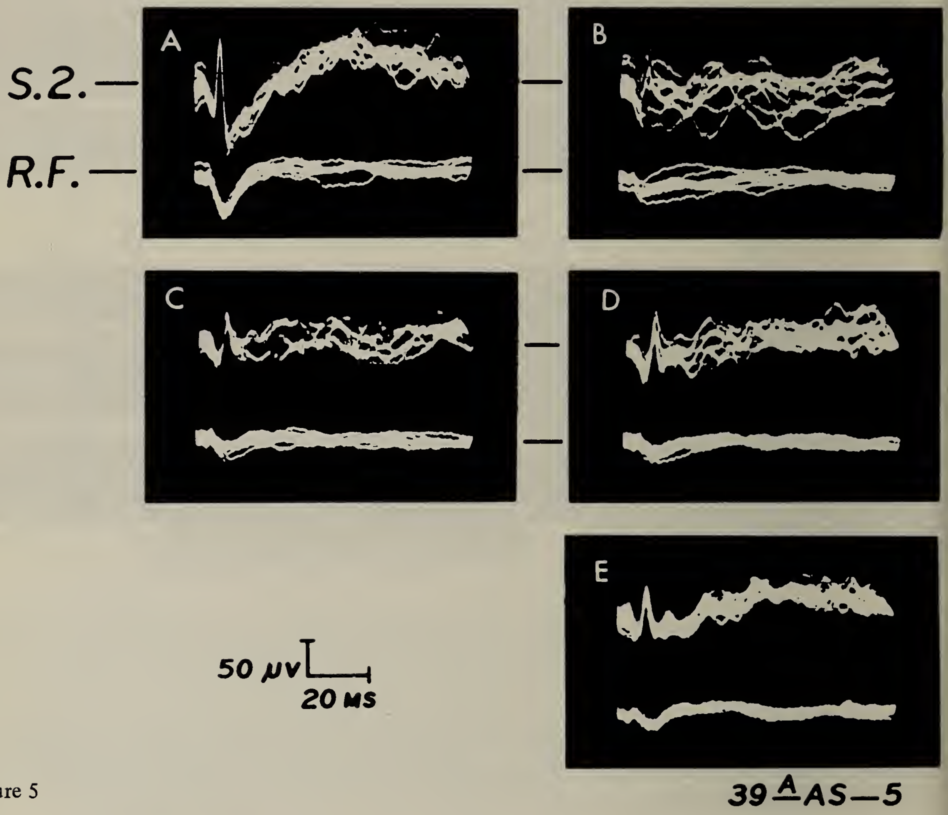

Figure 5

Evoked responses to electrical stimulation of the sciatic nerve recorded from the contralateral midbrain reticular formation (RF) and from cruciate fissure in the somatosensory cortex (S.2).

A, control; B, C, D, E, respectively 2, 15, 30 and 60 minutes after intravenous administration of alcohol (1 gm/kg). The results indicate a greater degree of depression in the reticular formation as compared with the somatosensory cortex (S.2). Reproduced from Schweigerdt *et al.* (1965). Nature (London) *208*: 688, with the kind permission of the Editor.

Dr. Domino: Is the primary initial positive wave still there?

Dr. Himwich: Yes.

Dr. Domino: So that one would conclude that the initial positive wave form which is mediated over the somatic pathways is still intact, but the negative is . . . .

Dr. Himwich: Is more depressed.

Dr. Domino: This is precisely what Story and French also report.

Dr. Himwich: In a different series of 14 cats, simultaneous recordings of the evoked responses in the midbrain (RF) and the cortical area

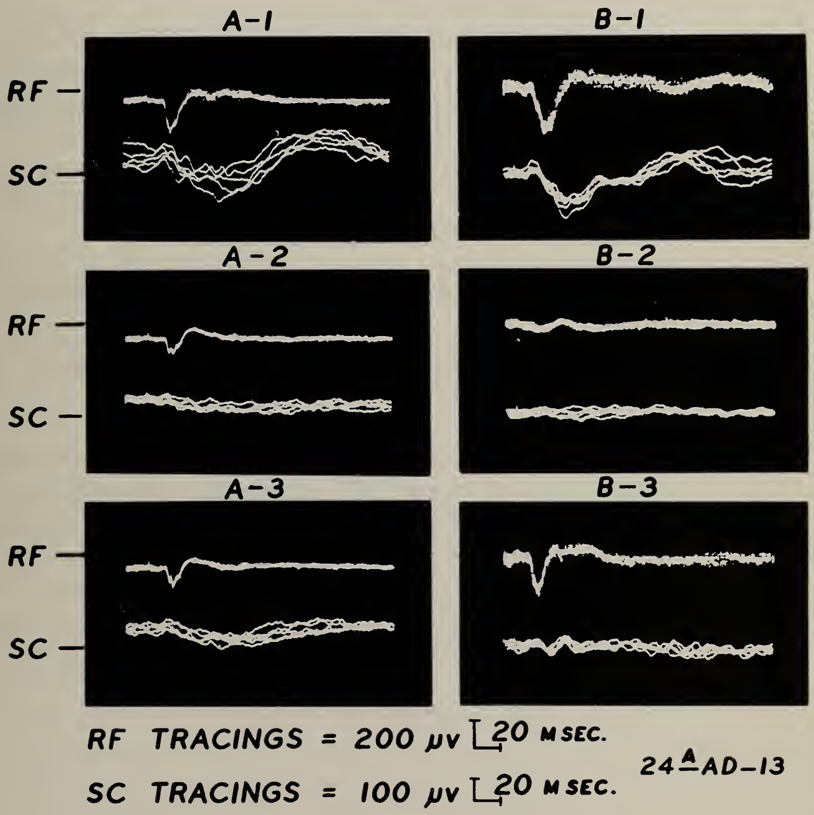

Figure 6

Effect of alcohol on the evoked potentials in the reticular formation (RF) and the somatosensory cortex (SC) upon stimulation of sciatic nerve. A-1, Control; A-2, 2 minutes after alcohol injection, a depression which was marked in SC and only partial in RF; A-3, 15 minutes after alcohol injection, onset of the recovery process which, however, was retarded in SC; B-1, control; B-2, 2 minutes after alcohol injection, marked depression of both responses; B-3, 1 hour after alcohol injection, marked recovery in RF but very little in SC. SC is the same as S.1 in Figure 2. Reproduced from Dravid *et al.* (1963). Nature (London) *200*: 1328, with the kind permission of the Editor.

designated as SC* on stimulation of the sciatic nerve, showed that in 9 of these animals, 2 minutes after alcohol, a marked depression of the response in SC was accompanied by a depression which was only partial in RF; 15 minutes after alcohol, recovery was evident in the RF response, but such recovery was retarded in SC. In 5 other cats, however, alcohol caused a severe depression in both responses, with marked recovery in RF but very little in SC, 1 hour after ethanol (Fig. 6).

Thus, it appeared that whereas ethanol caused a more profound effect on the response from the midbrain reticular formation than it did on that from the somatosensory cortical area near the cruciate fissure, the responses arising near the coronal suture were mostly *more* depressed than were those of the midbrain reticular formation.

Dr. Brazier: These are not long-living cats?

Dr. Himwich: No. All experiments were acute.

Dr. Brazier: So you do not know if you can get the same thing on the same cat twice?

Dr. Himwich: No, I do not. In an effort to try to solve the discrepancy between the contradictory relationships of the reticular formation to these 2 cortical sites, we performed additional experiments in which we studied both cortical areas simultaneously, with recordings made by means of both the kymographic camera and the CAT**. These revealed that the responses from both recording sites were depressed by alcohol, but a more marked degree of depression was seen in S.1 as compared to S.2 (see Fig. 7 for a representative experiment, recorded with the CAT). By the end of one hour of recovery (record E of this figure) S.2 exhibited practically the same amplitude as the control (record A), while S.1 was still visibly depressed in comparison with its control. Thus, this area (S.1) seemed more vulnerable to alcohol than did the second site (S.2). With the CAT, the greatest degree of depression observed at any time during the hour following the administration of alcohol, was more marked at S.1 than at S.2 in 4 of 6 instances, whereas with the camera, which gave more uniform results, the greatest depression occurred in S.1 in all 6 observations.

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* SC in this series of experiments corresponds to S.1 in the other experiments reported here.

**CAT = computer of average transients.

The extent of recovery at the end of one hour as observed with the CAT was greater at S.2 in 3 of the observations, equal at S.1 and S.2 in one, and greater at the S.1 site in two cases. With the camera, on the other hand, in 5 of 6 observations, the recovery was greater at S.2 (see Table II).

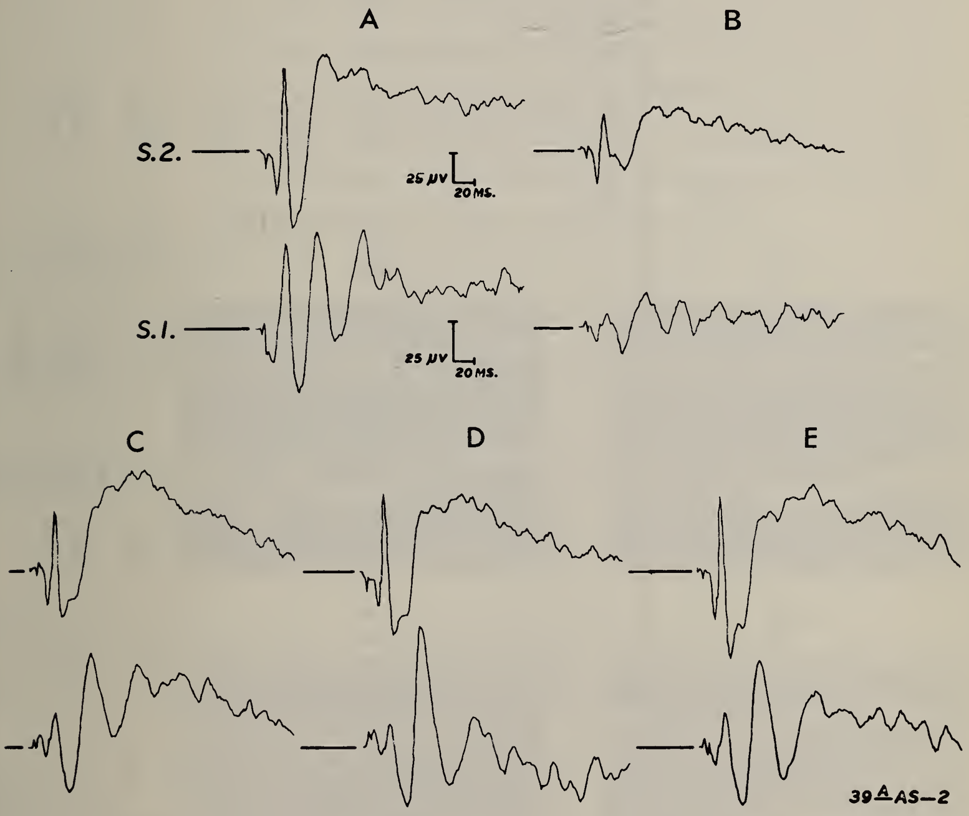

Figure 7

Evoked responses to electrical stimulation of the sciatic nerve recorded from the two cortical sites, (S.2) and (S.1), channeled through the CAT (computer of average transients).

A, control; B, C, D, E, respectively 2, 15, 30 and 60 minutes after intravenous injection of alcohol (1 gm/kg). The evoked potentials from S.1 yield rather variable responses while at S.2 they are of a more consistent form. The responses from both recording sites are depressed by alcohol, the effect being more severe in S.1 than in S.2. Reproduced from Di Perri *et al.* (1968). Quart. J. Stud. Alc. *29*: 20, with kind permission from the publisher.

TABLE II

Comparison of CAT and Camera Results *

CAT RESULTS						KYMOGRAPHIC CAMERA RESULTS					
Experiment	Site	Control Amplitude	$\frac{S.1}{S.2}$	Greatest Depression	Recovery at 1 hr.	Experiment	Site	Control Amplitude	$\frac{S.1}{S.2}$	Greatest Depression	Recovery at 1 hr.
#1	S.2	115 µV		45%	55%	#1	S.2	150 µV		35%	60%
	S.1	45 µV	.40	55%	55%		S.1	140 µV	.95	55%	45%
#2	S.2	110 µV		45%	95%	#2	S.2	155 µV		30%	100%
	S.1	75 µV	.70	75%	45%		S.1	150 µV	.95	55%	60%
#3	S.2	115 µV		55%	70%	#3	S.2	285 µV		45%	85%
	S.1	60 µV	.50	65%	50%		S.1	150 µV	.55	55%	75%
#4	S.2	125 µV		55%	50%	#4	S.2	150 µV		40%	60%
	S.1	55 µV	.40	35%	90%		S.1	80 µV	.55	60%	100%
#5	S.2	100 µV		60%	45%	#5	S.2	100 µV		40%	80%
	S.1	40 µV	.40	40%	60%		S.1	100 µV	1.00	50%	65%
#6	S.2	95 µV		65%	100%	#6	S.2	90 µV		45%	100%
	S.1	100 µV	1.15	75%	70%		S.1	80 µV	.90	50%	75%

*For explanation, see text, page 123 of General Discussion.

The evoked responses from 2 other discrete cortical projections were compared with those of their subcortical components and here again we found a difference in the relative effects of alcohol on the responses at these points. In experiments in which the evoked responses to photic stimulation were simultaneously recorded from one lateral geniculate body (LGB) and from the homolateral visual cortex (V.Cort.), it was found that alcohol caused only slight, if any, depression of the responses recorded from the former, whereas at the cortical level the later elements of the response were affected more readily and for a longer duration, while the primary ones showed only slight, if any, depression. No effect was exerted on the latency of the responses. Thirty minutes after the injection the cortical depression had been reversed to a great degree (Fig. 8).

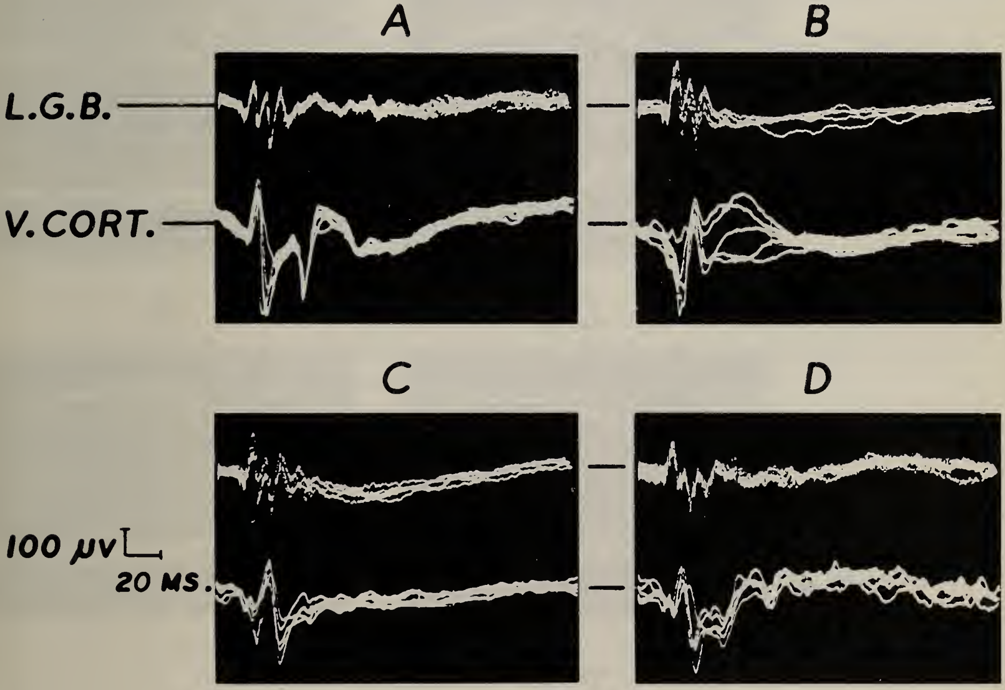

Figure 8

Evoked response to photic stimulation recorded from one lateral geniculate body (LGB) and homolateral visual cortex (V. CORT.).

A, control; B, C, D, respectively 5, 15 and 30 minutes after intravenous injection of alcohol (1 gm/kg). Depressant effect of alcohol is seen on the later waves of the cortical response which, however, are partially recovered 30 minutes after alcohol treatment (D). The LGB was hardly affected by this dosage of alcohol. In these traces the onset of the stimulus and the beginning of the oscilloscope trace are concomitant. Reproduced from Di Perri *et al.* (1968). Quart. J. Stud. Alc. *29*: 20, with kind permission of the Editor.

Dr. Hoff: The lateral geniculate body was less affected in the beginning?

Dr. Himwich: Yes. On the other hand, the effect of alcohol on the auditory evoked potential in the primary auditory cortex and in the inferior colliculus included a certain degree of depression in both responses, but the cortical response recovered 60 minutes after injection, while the collicular response was still partially depressed at that time (Fig. 9). In some of the experiments, however, both the inferior colliculus and the auditory cortex were depressed more or less equally

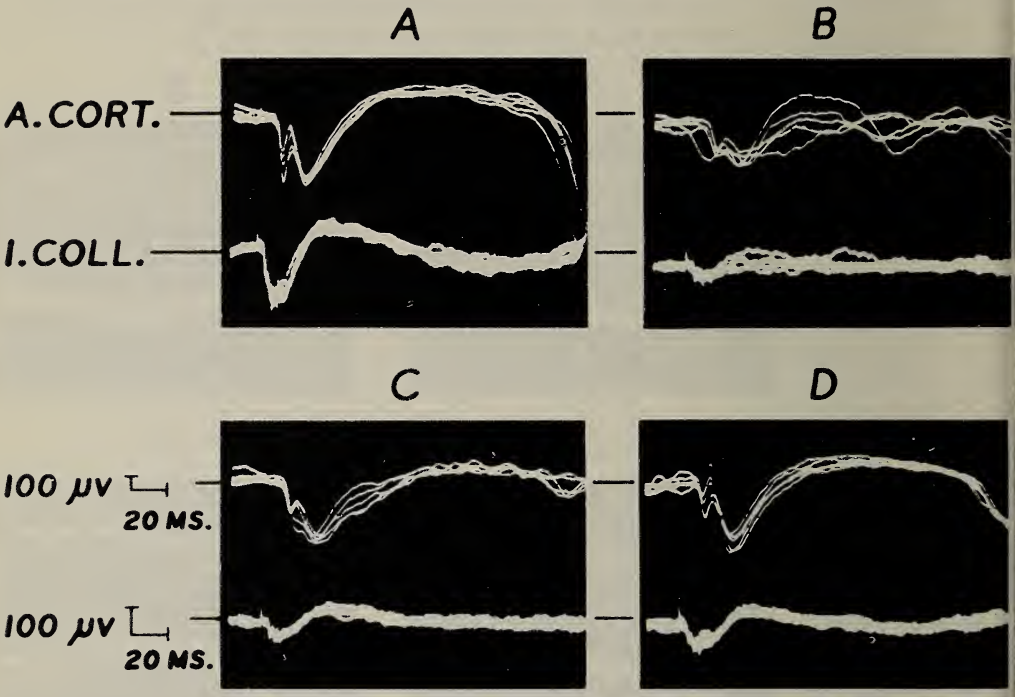

Figure 9

Evoked response to auditory stimulation recorded from the primary auditory cortex (A. CORT.) and from the homolateral inferior colliculus (I. COLL.).

A, control; B, C, D, respectively 5, 15 and 60 minutes after the administration of ethyl alcohol (1 gm/kg). The cortical response is fully recovered 60 minutes after alcohol treatment while the inferior colliculus still showed marked depression. Reproduced from Di Perri *et al.* (1968). Quart. J. Stud. Alc. *29*: 20, with the kind permission of the Editor.

Dr. Domino: Would you not want to compare this to the medial geniculate response, rather than to that of the inferior colliculus?

Dr. Himwich: We were not able to report the medial geniculate response because of its variability and rapid disappearance.

Dr. Brazier: Was it a click stimulus?

Dr. Himwich: Auditory stimulation was applied through a pair of earphones set about 2-3 cm. from the ears and activated every 2 sec. by rectangular pulses of 0.1 millisec. duration and 10-15 volts intensity, derived from a Grass S-4G stimulator.

Dr. Hoff: What electrode did you use for picking up the evoked potentials?

Dr. Himwich: It depended on the site. For example, the auditory cortex and visual cortex and the 2 sites in the somatosensory cortex were placed under visual inspection and we put the electrode at the place where we got the most consistent response.

Dr. Hoff: With needle electrode?

Dr. Himwich: No; macro-microelectrode. For the other site, we used the Horsley-Clarke, and the electrodes were placed in the standard way.

Dr. Domino: Dr. Brazier brought out the point that you are using different animals for your comparisons and therefore the electrode placement may be slightly different. I wonder if in some of your animals the recording site in reticular core might be close to the primary sensory pathways? Are you picking up more from collaterals of that system and, as a result, could it be that what you are suggesting as differential sensitivity within the reticular core is simply related to differences in electrode placement with respect to the classical sensory pathways?

Dr. Himwich: Well, I do not think it is so much a question of different sites in the reticular formation obtained by our Horsley-Clarke apparatus. We placed them as repeatedly as possible in accordance with our coordinates. But we know definitely that we had a few millimeters between the two sites in the primary somatosensory cortex; the differences between results in the primary somatosensory area, accessory somatosensory and reticular formation are of such an order that we do not think that they can be ascribed to differences in the placement of the reticular electrodes.

Dr. Domino: I wonder whether you should not use chronic animals, so that you can compare more precisely?

Dr. Brazier: I would suggest two things. If I understand your preparation correctly, for the experiments in which you stimulate the radial nerve, the animal has been prepared under ether and is paralyzed. Now every time you introduce that excruciatingly painful stimulus, you put its reticular formation into a different state than when you give it a click or a buzz in the ear or a flash in the eye. In other words, you are doing something really terrific to the reticular core, apart from recording whether or not it can transmit, or whether or not it is affected by alcohol. You have got an animal experiencing intense pain.

Dr. Himwich: We were studying 3 different modalities – the somatosensory cortex, the auditory cortex and the visual cortex – and the stimulation we used was as close as possible to minimal to obtain consistent evoked responses from the various sites.

Dr. Brazier: Two of the stimuli are painless and one is painful.

Dr. Himwich: That is correct; but even in the case of the painless ones we found two different kinds of relationship between cortical and subcortical areas in regard to auditory and visual functions.

Dr. Brazier: If I may make one other remark about the use of the CAT, the so-called 'computer of average transients' – as you know very well, it does not average; it has no circuits inside that can average. All it does is add. Unless you have exactly the same 'n' in your samples, you cannot make a justifiable comparison.

Dr. Himwich: I can add fuel to the fire you have just started. For example, we have found discrepancies between the amplitudes obtained by the camera and the CAT. In contrast to the line copies made by the CAT, the camera usually describes a band, because the 5 sweeps are not completely superimposable. In measuring the amplitude, the investigator chooses the highest point formed by the

5 records as the peak and the lowest point as the trough. Such amplitude measurements tend to increase the height of the peak and the depth of the trough. In contrast, the CAT does not record extremes but, rather, intermediate values for both the peaks and the troughs. Because of these different criteria, the measurements of the amplitudes with the CAT usually yield lower values than those with the camera.

Greater Ethanol Effect on Association Areas

Dr. Domino: There is one thing, Dr. Himwich, that bothered me. That is the cortical placements in your experiments. I believe that what you have denoted as S.2 is primary somesthetic cortex, as you have indicated, but your other point of recording is, I think, somesthetic association (S.1). In other words, I think you are into the lateral gyrus, which is association cortex.

Dr. Himwich: We based our topographical nomenclature on the paper of Marshall, Woolsey and Bard (1941), who considered both of these sites to be primary somatosensory.

Dr. Brazier: I think they were working before the days when there was real knowledge of what the projections were to these two sites – before it was known that, further posterior, the projection is through the non-specific system rather than through VPL*. I, too, feel strongly that S.1 is association cortex.

Dr. Himwich: I see the point that you have made, and I would agree that it is the association area from which we have been recording at site S.1**. That might explain the discrepancy between the two results, mightn't it?

Dr. Domino: If this is an association response, then I am also bothered, because recruiting responses, in contrast to augmenting responses, are not much blocked by ethyl alcohol. I think the fact that the response from the association cortex is also reduced is against this. What are the latency differences, or are they the same? Of course, it still could be that the non-specific systems are depressed.

Dr. Hoff: We are trying this out by using as our stimulus source the central cut end of the dorsal gastric branch of the vagus, and studying its afferent projections.

* VPL = nucleus ventralis posterolateralis of the thalamus.

**In subsequent publications (Himwich *et al.*, 1966; Di Perri *et al.*, 1968), the site S.1 near the coronal suture is referred to as the association area.

Dr. Himwich: I think some additional explanation should be made in regard to our results, which would underscore some remarks made earlier by Dr. Domino in regard to polysynaptic depression. In regard to evoked potentials, the somatosensory area S.1, which I shall subsequently call the association area, presents a greater polysynaptic development than the primary somatosensory cortex (S.2) and for that reason might be more readily depressed by alcohol. Perhaps the extent of the polysynaptic development of the midbrain reticular formation is intermediate between those of the 2 cortical areas examined. Amassian (1954) points out that the responses from the association area are more variable than those from the primary somatosensory cortex, an observation we have confirmed. Amassian further stresses the profound susceptibility of this area to even small doses of pentobarbital. This observation is especially interesting when considered in connection with that of French *et al.* (1953), who have shown that the reticular formation is relatively more susceptible than the somatosensory cortex to barbiturates as well as to ether, and we have extended their observations to include alcohol.

If the greater degree of depression depends upon the extent of the polysynaptic development, it is not surprising that the reticular formation is more depressed than the primary somatosensory cortex. Such an action on the part of alcohol may also account for the still greater sensitivity of the association area, not only in comparison to the primary somatosensory cortex but also to the reticular formation. In fact, of all the brain regions we studied, the association area was the most sensitive to alcohol. A similar explanation seems to apply to the transmission via the lateral funiculus and VPL nucleus to this area. The failure of alcohol to obtund the first spike of the lateral funiculus, as against the decreases in the amplitude of the second spike, may also be accounted for by a more complex synaptic neural pathway involved in the production of the second spike. For the same reason, the visual cortex is more sensitive than the geniculate body. Such a simplification however, cannot be applied to the relationship of the inferior colliculi and primary auditory cortical area.

We fully realize that the total behaviour of an organism in response to alcohol or to any other substance, depending as it does in large measure on the interactivities of many brain parts, is far too complex to be described in terms of action upon any single selected pathway or site in the brain. But it still may be pointed out that the overall behavioural response to alcohol reveals effects which appear to be most marked in the so-called higher functions of the brain; learning, judgment and self-criticism. Only with increasing dosages do we see the depression extending down the brainstem to lower areas and finally enveloping the vital centers of the medulla oblongata resulting in the production of shock. The earlier, more profound and, apparently, more enduring effects are exerted on cortical rather than subcortical structures in accordance with the clinical viewpoint that depression extends from above downwards along the neuroaxis.

Behavioural Implications of Physiological Effects

It is of some interest, therefore, to see to what extent our results may fit into the ideas of selective sensitivity in various fragments of behaviour. In the stimulation of the radial nerve we may expect that the sensory modalities of the upper extremity are involved, both deep and superficial, including that of pain. In view of the fact that the lateral funiculus and the thalamic VPL are depressed to lesser degrees than the primary somatosensory cortical area, we are led to speculate that the discrete awareness of pain and other modalities would be affected more profoundly than the more crude and diffuse consciousness of extreme discomfort, depending perhaps, in large part, on the function of the thalamus. It may also be pointed out that the primary somatosensory cortex proved to be less sensitive than the reticular formation and that this finding is in accord with the observation of Caspers (1958), though he used rats, employed different methods and studied other parameters. Kalant (1961) has presented clearly the behavioural implications of such a relationship.

On the other hand, comparing the reticular depression with that seen in the association area (S.1), the reticular formation was less depressed than the latter. Such diverse observations disclose relationships even more complex than previously seen. From what we know about the effects of anesthetics on the reticular formation, and alcohol may be regarded as an anesthetic, it might have been expected that the reticular formation would be more sensitive than the somatosensory cortex. Thus the impulses emanating from the reticular level may cease earlier than those from a cortical area which is not dependent upon this subcortical structure. But our data from only one of two cortical sites studied are in agreement with such a conception. Thus, comparisons between the degrees of depression of the reticular formation and the two different cortical sites reveal a complex situation in which some aspects of cortical contribution are more susceptible to alcohol than those of the reticular formation.

Similarly, with audition the evoked potentials from the inferior colliculus are as susceptible, if not more so, than those of the primary auditory cortex and in that case the hearing of sounds and their understanding would be lost simultaneously. But for vision the differences between the responses of the lateral geniculate body and the visual cortex support the concept of greater cortical sensitivity to alcohol so that discrete awareness of what we see might disappear before the function of seeing as such. If we refer to the concept of reverberating circuits and positive and negative feedback mechanism, we see that for some sensory modalities, but not others, some circuits with cortical components cease functioning before those which are represented chiefly by subcortical elements.

Dr. Kalant: It varies with the particular area.

Dr. Himwich: Yes.

Effects on Direct Excitability vs. Evoked Potentials

Dr. Kalant: May I ask one further question? In your work you studied only evoked potentials resulting from peripheral stimulation. Caspers' work, with which you have taken issue in the past, included a study of the response to direct local stimulation of the reticular formation and the cortex. If I recall his work correctly, he found that spontaneous electrical activity in the cortex continued with increased amplitude, in a synchronized pattern, under the influence of alcohol, while the spontaneous activity of the reticular formation was depressed immediately and progressively. On local stimulation, he found that the threshold for 'seizure discharge' was increased progressively in the reticular formation, while it was initially decreased in the cortex and then only increased at much higher alcohol levels. Does that give a different picture from what you concluded on the basis of evoked potentials?

Dr. Himwich: It is difficult for me to comment on this, because I could understand his results, but not his methods. I know his conclusions and I accept them.

Dr. Wallgren: Caspers' conclusions were based principally on the measurement of thresholds for direct local stimulation.

Dr. Hoff: Did he use alcohol?

Dr. Kalant: Yes, alcohol and barbiturates.

Ethanol and Disinhibition

Dr. Domino: I would like to wave the flag for more research on a related note. It is a common idea among scientists and laymen alike, that ethyl alcohol and barbiturates block inhibition and so cause a release phenomenon. I would like to stress that from the neuropharmacologic point of view there is not good evidence that alcohol depresses cortical inhibition. In fact, here is a set of words or sentences that everyone has been using for I do not know how many years, and yet somebody still has to prove them with research. Dr. Katsuda and I tried to study this by means of the phenomenon of surround inhibition which is mediated via the transcallosal pathway through the corpus callosum. One side of the cortex can influence the opposite cortex in such a manner as to facilitate precise symmetrical points, but inhibit surrounding points. We studied the effects of electrical stimulation off-centre, so to speak, in order to see how it affects a response on the opposite side, with the idea of utilizing the phenomenon of surround inhibition.

Evoked potentials were recorded from the cortex in response to sensory input such as stimulation of the median nerve. The recovery cycle was studied as it is modified by a conditioning stimulus to the opposite cortex off-centre. In this way one can get a very nice curve of recovery which should be indicative of a cortical inhibitory interaction. Barbiturates are extremely effective in blocking this influence of one side of the cortex on the somesthetic evoked response of the opposite side. Ethyl alcohol also depressed this effect, but at the same time it reduces the somesthetic evoked potentials (see Dr. Himwich's discussion pp. 116-132). In our own experiments, therefore, we have not been able to really prove that ethyl alcohol is selectively depressing cortical inhibition, but we have better evidence that barbiturates indeed do.

Inhibitory Neurons

Dr. McIlwain: Are there characteristic structural features if you look under a microscope at inhibitory neurons? Are these the entities which synapse on synapses? In that case can you perhaps recognize different parts of the nervous system by their different structural characteristics?

Dr. Domino: With regard to the existence of the Renshaw inhibitory neurons in the spinal cord, neurophysiologists can easily show their activity, but neuroanatomists are hard put to identify them. What these things look like under the microscope is really anyone's guess at this point.*

Dr. McIlwain: The virtue of having something that you can recognize as different under the microscope is that one can probably make an actual preparation of it by dispersion and differential centrifugation or some other mechanical means of separation.

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*Ed. Note: Structural differentiation of excitatory and inhibitory synapses was later reported by Uchizono (1965).

Dr. Brazier: Was one of your questions whether a synapse on a synapse has been seen?

Dr. McIlwain: Not only whether it had been seen; but I was wondering whether it was actually accepted as having a particular function.

Dr. Brazier: Sarah Luse (1963) of Washington University has reported seeing a synapse on a synapse with the electron microscope.

Dr. McIlwain: Yes; I think George Gray at University College, London, has also.

Dr. Hoff: A bouton on a bouton is not usual, though?

Dr. Brazier: No. I think Dr. Luse said she has found only one.

Dr. Hoff: Sometimes you see a bouton on the end of the dendrite. This may be what she has seen.

Dr. Wallgren: With ethyl alcohol, the only direct evidence for a selective effect on inhibitory pathways has been, I think, the work of Bernhard and Skoglund (1941) on the retina. Also, the Limulus eye has separate inhibitory and excitatory synapses, and there was higher sensitivity towards ethanol in the inhibitory synapse (MacNichol and Benolken, 1956).

Dr. Brazier: They gave the horseshoe crab some liquor?

Dr. Wallgren: Yes. They used an eye preparation and exposed it to solutions containing alcohol. But there is very little direct evidence. The observation by Bernhard and Skoglund was also supported by later work on the electroretinogram (Straub, 1957; Ikeda and Granger, 1963). But there is nothing about any part of the brain. Many people are very interested in these highly suggestive observations but no one except Dr. Domino's group has followed the matter up yet.

C. BEHAVIOURAL MEASURES IN LOCALIZATION OF DRUG ACTION

Dr. Russell: In our discussion so far the criteria of localization come at a level other than the behavioural level. I would like to make a few remarks on work which is relevant to the issue of localization and which has used *behavioural* measures as the ultimate end points or criteria.

As I emphasized in my paper, the behavioural scientist is particularly concerned with interactions between three classes of variables – drugs, neurochemical events, and behaviour. When he gets down to trying to answer questions which have to do with identifying neurochemical events that may be correlates of the behavioural measures he is studying, he very soon finds himself asking also *what parts* of the nervous system are involved? Thus, localization becomes an important issue very early in this work.

To try to clarify the localization, the behavioural scientist has used three types of approach:

1. Studies involving the use of tertiary and quaternary forms of drugs, taking advantage of their differential capabilities of penetrating the blood-brain barrier in order to compare peripheral versus central effects (comparisons of atropine and methyl atropine, for example).
2. Studies in which the drug is introduced into the cerebrospinal fluid by intracisternal or intraventricular injection.
3. Introduction of the drug directly into some particular site in the CNS via implanted micro-cannulae.

Let me illustrate the kind of relation that may be found between a particular neurochemical event in a particular site, and a specific effect upon behaviour. Grossman (1960) described what may be called 'neurochemical coding' of a specific form of behaviour: food and water intake. Using micro-cannulae permanently implanted into certain circumscribed regions of the hypothalamus, he was able to induce water-intake behaviour, in animals completely satiated for water, by the administration of the cholinergic drug, carbachol. On slight movement of the site of chemical stimulation, the behavioural response was not obtained. In the same site, in the same animal, via the same cannulae, injection of an adrenergic agent induced eating. His general conclusion was that in a circumscribed CNS site there exists neurochemical coding for specific forms of behaviour. Fisher and Coury (1962) used this same technique, as a means of mapping functional pathways through that general region of the brain. These findings reinforce a point that Dr. Dews raised earlier: we do not necessarily have to think of localization of a behavioural pattern in terms of one specific spot in the nervous system; it may involve pathways that wander through various parts of the nervous system, but it may be possible to trace the circuit by neurochemical stimulation in the way I have just described.

One interesting point about this series of studies is the fact that they were concerned with what, at a theoretical level in psychology, would be referred to as the construct of 'Drive.' This construct is particularly important when we are considering ways in which addiction behaviour may be maintained once it has been established.

Using Grossman's techniques, we have been examining the question of whether it is possible to support the acquisition of behavioural patterns of varying levels of complexity that normally are dependent upon peripheral mechanisms (deprivation of water), using direct cholinergic stimulation as a substitute for these mechanisms. We compared animals on 23½ hour water deprivation with animals which are completely satiated, i.e., maintained on *ad lib* water, but are stimulated by the introduction of carbachol into the hypothalamus just before the start of a learning session. Using water as the reinforcer for both groups, we studied the acquisition of operant responses, both simple and more complex, in these animals. We found that the behaviour can be turned on or off, by applying or withholding direct chemical stimulation, in the same way as by depriving or satiating the animal. If the water-deprived animal is satiated, he does not learn the task, or perform it if he has already learned it; if we inject normal saline rather than carbachol into the control animals, they will not acquire the task, or perform it if previously learned. Furthermore, if after the water-deprived animal has learned the behavioural pattern we then remove the water reinforcer, the response is extinguished very rapidly; if we do the same to animals under direct central carbachol stimulation, the behaviour is also extinguished very rapidly. Here, then, is an example of the effects of a drug in particular CNS sites affecting particular kinds of neurochemical events that, in turn, are reflected in control over behaviour patterns.

It seems to me that it is possible, using behaviour as the end point, to get some very useful information about localization of important drug effects. When drugs are given peripherally many systems within the body may be involved, yet one particular system may be of special importance for a particular form of behaviour. Localization, then, may occur in the sense that neurochemical events in a particular site or functional circuit may be related to a specific form of behaviour, even though the drug may have broader effects through its actions on other sites.

Dr. Brazier: I think this is tremendously important. Here are some areas in which the neurophysiologists might extend their observations on the localization of drive, if I may use the psychologists' word for the time being. There is a great deal of material now from other laboratories – for example, from the Nencki Institute in Warsaw, from Andersson's laboratory in Sweden, from Brobeck and others (see Brazier, 1963) – about areas in the brain which, if stimulated, will make the animal go on eating until it is more than satiated; the food falls out of its mouth but it still goes on eating. Or else you can stimulate it in another part and however hungry the animal is, it will not touch the food. And there is the thirst story which you have already mentioned. And there are Olds' (1958) experiments, in which stimulation of some areas of the hypothalamus will make the animal go on bar-pressing for what man interprets as being a pleasurable experience, while with the electrodes in other sites it will press to avoid what Olds has interpreted as pain.

Studies on Addiction

I wonder whether one could not think of addiction first of all rather broadly, and then neurophysiologically. In this way, perhaps one would be able to focus down on it more. Are there not far more divisions of subjective state than pleasure and pain, that one can conceptualize? For example, the avoidance of pain, which now appears to have some localization, at least in a rat, is perhaps related to the one part of the brain which is most involved with the addiction to morphine. The parts of the brain that are concerned, perhaps, with drives for increasing one's ability to deal with things might be related with the amphetamines or with marijuana. The parts of the brain that are concerned, on the contrary, with having to avoid dealing with things are perhaps involved with addiction to the sedatives. In other words, neurophysiologists might search in parts of the brain for much finer distinctions than just those which anthropomorphically are called pleasure and pain. These drives may perhaps have loci in the brain that relate very definitely to whether the addiction is to a drug taken to enhance action or to avoid having to take action.

Studies with Morphine

Dr. Dews: I endorse what you say very strongly. I think this is a desirable road to pursue. Unfortunately, I think we are further from being able to start on it than many are inclined to think. I would like to talk a little about morphine, which everybody knows is an analgesic, i.e., an attenuator of the effects of painful stimulation. If you look over the literature of the behavioural studies that have been done on morphine (and there are surprisingly few), dealing with animals in well controlled situations, you find, however, that the most characteristic thing that morphine does is to reduce behaviour. Whether you are dealing with behaviour maintained by food, by water, by electric shock or by anything you choose, morphine causes reduction in the behaviour. What is more, morphine causes reduction in behaviour whether it is food-maintained, water-maintained, shock-maintained or what have you, at approximately the same dose levels. The more comparable the actual behaviour you are dealing with, the more comparable are the dose levels that cause reduction in that behaviour, without regard to what is maintaining the behaviour (Kelleher and Morse, 1964).

The impression you come away with, very forcibly, is that the experimental evidence does not support the selectivity of morphine, in relation to specifically painful stimulation, nor even for anything that you might call unpleasant. You might say, so much the worse for the experimental studies; obviously the difficulty is one of extrapolation of these animal studies to humans. But if you look at the evidence for morphine having a selective effect on pain in man, you find, again, the evidence is poor. I am not saying that morphine does not reduce the unpleasantness of someone's pain sensations. There is no question that morphine can do so. What I am talking about is selectivity. Is the effect on pain a specific effect? Does the effect on the consequences of painful stimulation take place at dose levels, or on occasions, when other sorts of behaviour would not be reduced? We certainly know that morphine will alleviate non-painful situations in the human patient. In cardiac asthma, for example, where the patient is in no pain in the true sense of the word, he experiences great 'subjective' relief from a dose of morphine. If you give a dose of morphine to a connoisseur just before a magnificent meal, would he not become as uninterested in the meal as the patient in pain becomes uninterested in his pain? Does not morphine reduce sexual behaviour, and other forms of social behaviour in which there is no reason to suppose that there is any unpleasant component?

Morphine and Pain – Specificity?

There is one test, widely used in the laboratory, that does seem to distinguish morphine and similar analgesics from other drugs which affect behaviour. The test deals with the flick of the tail of a rodent. If you do the test properly, using thermal stimulation, you can show effects with morphine but not with, for example, chlorpromazine. But if you change this test a little bit, if you use electric shock instead

Tail Flick Test

of thermal stimulation, if you go to other forms of painful stimulation such as tooth pull or stimulation of the Gasserian ganglion, then you can detect no difference between morphine and chlorpromazine. It is generally assumed that it is the painful component of the stimulation to the tail that is attenuated, but if the morphine effect is selective for anything it seems to be more related to the heat stimulation.

Dr. Kalant: What does this test consist of?

Dr. Dews: The tail flick? You simply put a mouse (or a rat) on a stand under some sort of a restraint and lay out its tail along a groove. Part way along the groove there is a little hole and beneath the hole is an electric wire, through which you pass a current. The wire gets hot and the mouse flicks its tail. Following a dose of morphine of the order of 10 mg/kg, the mouse will leave its tail in the groove and allow it to be burned. Following reasonable doses of chlorpromazine the flick is not abolished. But if you use other forms of painful stimulation, chlorpromazine comes out as well as morphine. Furthermore, it has been shown that you can get the tail flick, and also the effect of morphine on it, in spinal animals. So this effect of morphine may not have anything very much to do with the effects of morphine on the brain.

Dr. Kalant: As an exercise in the undergraduate pharmacology lab, we use a test based on thermal sensitivity of the feet in mice. Again, chlorpromazine does not reduce the sensitivity, while morphine does.

Dr. Dews: That is right. If you stay with thermal stimulation, you can get this distinction. If you use electrical stimulation of nerves and things of that kind, then you do not get it. In Dr. McIlwain's terms, these are very highly evolved tests, because people in the drug houses for at least 30 years have been looking for tests which distinguish morphine from everything else. If you just go into the laboratory and dream up either a situation involving painful stimulation or a situation that has plausibility as involving anxiety, then morphine comes out like chlorpromazine.

Addictive Properties of Morphine and Chlorpromazine

The interesting distinction between morphine and chlorpromazine is that whereas morphine is the drug of addiction par excellence, chlorpromazine, I would say, is the drug of non-addiction par excellence. Years ago, it seemed to me that any drug which had a fairly profound effect on the central nervous system was liable to be dangerous as a drug of habituation. If you look over the list – the barbiturates, alcohol, the Datura alkaloids, cannabis indica, amphetamine, cocaine, caffeine – just about any behaviour-affecting drug you could mention has at some time in history, in some part of the world, been abused. It seemed that one could generalize that anything that had a profound effect on the central nervous system would be abused or potentially abused.

There is no question that chlorpromazine has very profound effects; but it is not abused. So that we have this paradox – two classes of drugs, morphine-like and phenothiazines, which in practically all behavioural situations in experimental animals, and, I suspect, most situations in humans, are more alike to one another than they are to any other classes of drugs. Yet, on the one hand, one comprises the most addicting drugs, and the other, the least addicting drugs. What is the crucial difference?

Behavioural Toxicity

Dr. McIlwain: This gives me an opportunity to ask a question I wanted to ask Dr. Russell yesterday. He used the term 'behavioural toxicity' and I was very unclear as to what was meant by this. Do you actually mean the situation Dr. Dews has just mentioned, i.e., if morphine will cause a rat to get its tail burnt, is this behavioural toxicity? Or are you thinking in other terms?

Dr. Russell: That is one of the questions I also had down to ask Dr. Domino, who raised the question earlier of what 'toxic' means, and whether the term ought to be used. Could a definition of 'toxic' from a behavioural point of view be given in terms of a shift from specific to non-specific action? Earlier I talked about absolute and terminal thresholds that define the specific action of a drug on behaviour. One definition of 'toxic' from a behavioural point of view might be in terms of terminal threshold, defined by a shift from specific to non-specific action.

The term 'behavioural toxicity', as it was coined and first used, referred to decrements in behaviour. This basis of definition presents real problems. Are *all* decrements to be included? If not, what are the criteria for differentiating those that should be included from those that should not? What, indeed, constitutes a decrement? Does this use of the word 'toxic' have the same general features that the term has when used in other disciplines?

I would agree with Dr. Domino's statement that we ought to be very careful in the use of the concept, and particularly so when applying it to behavioural phenomena.

Dr. Kalant: Does this not involve a circular argument? Dr. Dews has just pointed out that with a drug like morphine, it may be very difficult to define a specific effect. Therefore, the meaning of 'specific', in terms of an experimental study, would depend upon the particular behavioural criterion chosen for the study. The transfer from one particular modification of behaviour to a more diffuse one might be a quantitative matter of dose-response relationship that would be paralleled if you had picked any other single behavioural deficit to study as your first test. What would be your criterion for specific and non-specific effect?

Measure of Behaviour to be Chosen for Study

Dr. Russell: Your point is well taken and, it seems to me, again raises questions about the search for behavioural modes of action of drugs. From the behavioural point of view, we should direct attention toward specifying whether a drug is having its effect not upon some *ad hoc* behavioural measure, but rather upon basic features of behaviour. The effects should be defined in terms of a series of carefully chosen behavioural measures which would enable us to say that the drug affects sensory input, or motor output, or central cognitive factors, or central motivational factors, or central emotional factors. These terms – motivational, cognitive, emotional, etc. – are basic constructs in any theoretical model of behaviour. In order to study these, it is necessary to have a battery of behavioural measures, some of which are directed toward providing information about sensory effects, others about motor effects, etc. Miller's (1956) efforts to define effects of certain drugs upon motivation are examples of this approach.

Suppose that we have such a profile of measures, and that we find that at a particular dose level significant visual impairment occurs, but other aspects of behaviour are not affected. And suppose then that we increase the dose and find that impairment appears in the other behavioural measures. Clearly, a shift has occurred from a specific behavioural mode of action to a generalized non-specific effect of the drug. 'Behavioural toxicity' might be defined in terms of such shifts.

Dr. Himwich: Clinicians have an unfortunate idea of what behavioural toxicity is. All the actions of the drugs which they desire for their patients, they regard as *the* actions; any other effects of the same drug which they do not desire, they refer to, in paper after paper, as behavioural toxicity.

Dr. Dews: Yes, I think we are getting on very shaky grounds here. In a simple example, where you take a barbiturate and you get agranulocytosis, then there is no question about it – agranulocytosis is a toxic effect. It is a different sort of effect. But I think it would be unfair to talk about death through Stage IV anesthesia following barbiturates as a toxic effect of the barbiturate. This is an extension of the therapeutic effect. It seems to me the only touchstone we can have is the one Dr. Russell suggested; toxicity involves something additional to the primary effect you are dealing with. For example, thiopental is a dangerous drug (in unskilled hands) because overdosage is easily achieved, but chloroform is a toxic drug because of its hepatic effects, not its primary effects on the CNS.

Environment as a Factor in Behavioural Toxicity

Dr. McIlwain: I am still curious as to what makes behavioural toxicity in distinction to other forms of toxicity. As far as I can see, although we have talked around this, I do not think the question has been answered. Is it not that in behavioural toxicity, one is involved more with the environment? In judging ordinary toxicity, one is practically considering only the animal and the drug. In judging behavioural toxicity, one is concerned with the drug, the animal and the environment. To take a particular instance, the tail-flick test, chlorpromazine would not be a damaging agent in the presence of heat. In another situation chlorpromazine might be toxic where an animal is put into a cold environment, because its body temperature would fall and it would die in hypothermia, whereas if the body temperature were maintained, it would not. This is another category of toxicity which deals much more with the influence of the environment and here, of course, we come closer to the problems of addiction because in this connection we are continually considering the social environment.

Dr. Russell: You are right in the sense that all measures of behaviour involve an interaction of an organism with an environment, whether it is his natural habitat or some test environment set up for experimental purposes.

Dr. McIlwain: In many neuropharmacological methods one seeks to be independent of the environment and may conclude that one has a real action of a drug when an effect is obtained under a variety of different environmental conditions.

Dr. Dews: To extend the term ‘toxicity’ to include undesired but primary effects in particular circumstances is unwise, because what is considered undesirable in one set of circumstances and one community may seem desirable to another set of people in another place of residence.

Cultural Reactions In Behavioural Toxicity

We have the difference between opium and alcohol in the United States as opposed to traditional China. In China alcohol is an exceedingly 'behaviourally toxic' drug. It makes people talk a lot and be disrespectful to their superiors – most undesirable behaviour; whereas opium makes them peaceful, quiet and cooperative. In the United States, alcohol is beneficial when it helps people be extroverted, the life of the party, while opium just lulls them into a dull monotony, and is therefore considered clearly 'behaviourally toxic.' I think that as soon as we get away from a definition based on types of action that are pharmacologically dissociable from one another, we are on very difficult ground. Whether one should call the Parkinsonian syndrome caused by phenothiazines a toxic effect or not, according to this definition, depends on whether you can show that it is dissociable from the therapeutic effects. Thus it becomes an experimental rather than a moral question. If it is dissociable, then you can call it toxic, if it is not, you cannot. You just leave the question *sub judice* until it is solved.

Dr. Brazier: I think we have left out a classification. That is the one for certain drugs – Jamaica ginger is an example – which produce sublethal but irreversible changes on the nervous system.

Drawbacks in Term 'Behavioural Toxicity'

Dr. McIlwain: So that if one produces irreversible change in the sense that the rat burns his tail, that is ordinary toxicity. It becomes behavioural toxicity when one induces some new pattern of behaviour which is undesirable. But what is toxic behaviour in effect? I would like to have it specified.

Dr. Russell: As far as I am concerned, I prefer not to use the term. Where it is used, I have seen it applied in both the ways that I have described: one, in the sense of differentiating between specific and non-specific effects upon behaviours; and the other, used to refer to any decrement in behaviour.

Dr. Kalant: Perhaps the answer is to avoid the use of the word 'toxicity' altogether, and refer only to specific impairment at minimal doses and diffuse impairment at higher doses.

Dr. Russell: It would not, I believe, handicap us very much in describing and discussing the behavioural aspects of the drug to leave out the word 'toxicity.'

Dr. Domino: We all agree 'Behavioural Toxicity' is a good lay word.

Dr. Quastel: I think it is similar to the term 'side effects.' I remember a statement of Peter Ustinov – do you remember, he said that when he took a drug, the chief reactions that he experienced were the side effects!

Dr. Kalant: The question of side effects, again, is a matter of what action you want or do not want. Dr. Himwich pointed out that a side effect is what you *do not* want, even though it may be part and parcel of the same basic action. For example, in some antihistaminics the same chemical grouping gives rise to antihistaminic effect, sedating effect, and some quinidine-like effect on the myocardium; you can call whichever one you want in a particular case the therapeutic effect, and the others are called side effects.

Specificity from Behavioural Viewpoint

Dr. Domino: I would like to come back to the issue raised by Dr. Dews regarding the word 'specific.' I believe he said that morphine is non-specific in that it produces behavioural depression generally, irrespective of whether one is using food, water or shock as the motivational stimulus. But the word 'specific' depends on your point of view; I would like to think of the actions of morphine and chlorpromazine, for example, as highly specific compared to the barbiturates, meprobamate, chlordiazepoxide or ethanol. I wonder if Dr. Dews would agree that we are simply dealing with relative degrees of specificity, in that barbiturates, for example, are rather non-selective in blocking conditioned as well as unconditioned responses, whereas chlorpromazine and morphine more selectively block the conditioned responses a little more than the unconditioned.

Morphine as a Reinforcer

Dr. Dews: Oh, I did not in any way mean to imply that morphine is not specific. I think it is an *exceedingly* specific drug. What I am saying is that it is not specific in regard to unpleasantness in the way that the traditional pharmacological textbook would have us believe. It may be that morphine is very highly specific as a universal reinforcer. It reinforces its own administration very effectively, because people go on taking it. It also supersedes, so to speak, all other reinforcers, so that in extreme addiction you are no longer reinforced by water, even though partially deprived of water; you are no longer reinforced by food; you are no longer reinforced by sex; you are reinforced by morphine. It takes over, so to speak; it becomes the ubiquitous reinforcer. I would consider this an action of very great specificity.

Let me give an analogy. If you take somebody in heart failure and measure the blood flow in all different parts of the body, and then give him digitalis, you find that blood flow goes up in just about all parts of the body. You would not say that therefore this is not a very specific drug. It is just that the differentiation between blood flow in arm and leg and head is not where you should be looking for the specificity. And what I am saying is that from all we know, the selectivity of morphine is upon the well-springs of behaviour rather than upon the kind of behaviour or the kind of reinforcer that is involved.

Morphine and Stages of Behaviour Pattern

Dr. Russell: Would another way of saying this, perhaps, be that specificity from the behavioural point of view may be defined in terms of basic features of behaviour rather than very specific measures of behaviour? Morphine may very well depress behaviour generally. But this does not really tell us what its behavioural mode of action is. It stresses the point that, in trying to define drug action, a single measure of behaviour is just about as useless as a single dose of a drug, or measurement at a single time after administration. Therefore, it is important for us to use batteries of behavioural measures that are selected for a particular purpose, either in terms of a theoretical model of behaviour or in terms of an empirical analysis of a wide range of behaviour, using factor analytic procedures or one of the several other methods for selecting behaviour patterns to be studied that I outlined yesterday.

Dr. Domino: Dr. Russell, you stressed acquisition, maintenance and extinction as three separate categories of learned behaviour. Certainly one would postulate that if morphine depresses drive mechanisms, acquisition should be far more selectively blocked by morphine than any other of these states. Is this true?

Dr. Russell: No, I do not think that this necessarily follows. These are three stages in the natural history of a particular behaviour pattern. Acquisition, maintenance and/or extinction might all be influenced by anything that would generally affect the drive involved.

Dr. McIlwain: But it is attractive to have a characteristic such as drive picked out in this fashion, which is something exhibited only in terms of behaviour rather than in terms of simpler systems. This is approaching a behavioural characteristic which is specific to your level of information. But you seem to regard this as a little unreal until there is a neurological basis for it. Now is this a correct view?

Study Neurochemical Events Intervening between Drug and Behaviour

Dr. Russell: No. I do not mean to imply this. It certainly is possible to study the effect of drugs on behaviour without being concerned with events that go on in the 'black box.' But it seems to me that, as psychopharmacology develops, it becomes very important for practical as well as theoretical purposes to get into the black box and find out what does intervene between the administration of the chemical stimulus and its effects upon behaviour. I do not know whether all the clinicians in the field will agree with me, but I think that, as far as psychoactive drugs and chemotherapy for mental illness are concerned, we have been on a plateau for some time. I cannot see how we are going to get off that plateau until we know more about these intervening events, and can begin to select or to synthesize drugs that act upon specific neurochemical systems known to be related to basic features of behaviour.

Characterize Behavioural Effects Themselves

Dr. Dews: In terms of ultimate and noble objectives, we want to know what drugs do and how they do it, at all levels and in all systems, down to the levels of cellular biology and the subatomic mechanisms of molecular interactions. It seems to me that the big stumbling block for further progress in understanding morphine at the moment is not the need for more neuropharmacological analysis of what have been considered its salient behavioural effects. What is needed is a systematic investigation of the behavioural effects themselves, so that sound guide lines can be given to neuropharmacologists as to the sort of functional changes in the brain to concentrate on, in looking for the underlying neurological mechanisms of the characteristic effects of morphine. It seems to me that until we can characterize what are the changes caused by morphine in functions of the brain, by which I mean in the input-output relationships, we are unlikely to be able to find out how the changes are mediated neurologically.

The neuropharmacologist is faced with innumerable sites and paths and directions for study, and the chance of stumbling on the right track is remarkably small. Even if it were stumbled on, it would not be recognized. If we can tell him that morphine characteristically affects a unique reinforcing mechanism, then he can start to bear down on it. If, on the other hand, he is looking for a specific effect on pain mechanisms, which morphine in fact does not have, then the probability that he will be successful in this search is quite low. If we can get the neuropharmacologist started down the right track in describing the mechanisms for a particular characteristic behavioural effect of the drug, then the effort in giving him this characterization will be amply rewarded by the increased depth of understanding we can get from the mechanism. This in turn will reflect into greater understanding of the behavioural effects and suggest new experiments in this field. I agree with Dr. Russell that we are on a plateau and it seems to me, if I may mix my metaphors, that one thing we could do to break the log jam and get off this plateau is to characterize in more precise functional terms what these drugs are doing to the behavioural systems.

Dr. Kalant: Is this not even more true when one considers that the various drugs we have been talking about have many areas of overlap in their actions, and that any hope of separating their effects depends on progressively more precise behavioural differentiation?

Dr. Russell: Dr. Dews' point and mine are not antithetical. They may involve a slightly different emphasis, but I would certainly agree with him. I am unhappy about what has happened since 1957, when psychoactive agents started to become very popular in the treatment of mental illness. Many researchers went into psychopharmacology without having adequately considered issues of methodology and without searching for an adequate basis upon which to conduct the analysis of drug effects. I think this is one of the points that Dr. Dews is emphasizing: that behavioural scientists need to look much more carefully at what I have been calling the behavioural modes of action of drugs, rather than merely taking one measure of behaviour with one drug, frequently given at only one dose, and measured at only one time after administration. There are far too many such studies in the literature.

Significance and Interpretation in Terms of Orientation

Dr. Brazier: I think I understand what you have said. The message has come through several times, and from Dr. Dews too, that the difficulty about all of us as experimentalists is that we are set to look for something specific, and we observe *that* when it is there; but there may be something else important going on that we miss because we are set in this particular way. For example, the pharmacologist who heard the fascinating story about the rat's tail (p. 138) no doubt thought, well, this is an extremely interesting point about morphine; but to me, it was an extremely interesting point about the nervous system, about thermal impulses, because that is my orientation.

Dr. Domino: Dr. Dews has emphasized the difficulty in differentiating between the effects of morphine and chlorpromazine. What is the situation with regard to alcohol, barbiturates, meprobamate and chlordiazepoxide? Can these be distinguished in terms of behavioural tests?

Drug Distinction by Behavioural Tests

Dr. Russell: I do not recall an experiment in which they have all been used with a battery of behavioural measures which would give an adequate profile of comparative behavioural effects. However, Janssen, in discussions at a Ciba Foundation Symposium (Steinberg *et al.,* 1964), reported that his profiles, derived from a battery of behavioural measures, were sufficiently precise to enable him to speak of atropine-like, morphine-like, and chlorpromazine-like drugs. It appeared, further, that he could extrapolate from these animal studies to the effects of those drugs in man.

Dr. Dews: I think that in experimental animals and in man there is a surprising degree of similarity between barbiturates, meprobamate and chlordiazepoxide. At low dose levels the response curves seem to go along nicely together. However, the dose-effect curve of meprobamate starts levelling off, whereas increasing doses of barbiturates will produce real anesthetic effects, which are never seen with the other two. In the present state of our knowledge it seems to me that the most parsimonious hypothesis is that meprobamate, chlordiazepoxide and barbiturates have certain properties in common, but that barbiturates have additional actions which come in at higher dosage. There are quite a number of different test situations in which barbiturates, meprobamate and chlordiazepoxide give essentially similar effects. Chlorpromazine, morphine and other compounds are quite different.

In avoidance situations, chlorpromazine reduces responding in doses at which the animal is still fully coordinated, while barbiturates, meprobamate, and chlordiazepoxide do not. If you work with most schedules of positive reinforcement, chlorpromazine generally – in most extensively studied species except the pigeon and perhaps the cat – tends to reduce responding. Barbiturates, meprobamate and chlordiazepoxide quite characteristically, over quite a substantial dose range, increase responding under an appropriate schedule of positive reinforcement. So here barbiturates, meprobamate and chlordiazepoxide are falling into the same category again, in distinction to chlorpromazine and morphine.

If you go to situations involving behaviour which is maintained by positive reinforcement, but in which responses are also sometimes simultaneously punished by aversive stimuli contingent upon the responses, responding can be greatly reduced when the punishment is in effect. Under these circumstances if you give morphine then responding goes down, first in the punished periods and then, with larger doses, also in the non-punishment periods. If you give chlorpromazine, the same things happen. If you give barbiturates, meprobamate or chlordiazepoxide, then very characteristically and very dramatically, you get an increase in the punished behaviour (Geller and Seifter, 1962; Kelleher and Morse, 1964).

Over all these different varieties of situations, barbiturates, meprobamate and chlordiazepoxide come together, and are clearly distinguished from chlorpromazine and morphine. The main differences that we know of between barbiturates, on the one hand, and meprobamate and chlordiazepoxide, on the other, are in the slopes of the dose-effect curves (those for barbiturates being steeper) and in the production of anesthesia by barbiturates at higher dose levels.

Dr. Domino: I suppose ethyl alcohol would be in the same category?

Dr. Dews: I do not know.

Dr. Domino: The statement has been made that meprobamate is 'ethyl alcohol in pill form.' Do you agree with this?

Dr. Himwich: I am not sure. I know that alcohol has been used experimentally for general anesthesia in Mexico.

Dr. Domino: You can get coma with meprobamate, too.

Dr. Himwich: But there is still a difference, in my opinion. With alcohol the anesthetic dose is very close to the toxic dose, while with meprobamate the doses are not close.

Dr. Dews: I might also add that meprobamate, like barbiturates, is being abused, whereas chlorpromazine is not.

Dr. Kalant: And withdrawal symptoms have been reported with meprobamate, too (Essig, 1966).

Ethanol and Behavioural Tests

Dr. Russell: Ethanol, is, in some ways, an interesting drug for illustrating some of the characteristics of drug-behaviour interactions. There is evidence for a threshold in blood alcohol level below which behavioural effects do not appear, although this level is very low. Above this level, effects differ for different forms of behaviour: more complex behaviour, mediated by higher brain centers, is likely to be affected at lower levels and to a greater extent than simpler components of behaviour. Indeed, some research results in man have suggested that simple reaction time, for example, may be shortened after small doses, whereas discrimination or choice reaction time is increased. Similar research has also been done with infrahuman animal subjects.

As dose level increases, even those responses that are initially enhanced begin to show decrements; the relation is not monotonic. Examination of time-response data shows that the shape of the curves for these behavioural effects is similar to the shapes of curves for blood and urine alcohol levels following administration of suprathreshold doses, suggesting that the behavioural effects are related to the presence of the drug within the body. One of the best studies concerned with relations of the kind I have been describing was conducted by Drew and his colleagues (1959), using a particularly good research design (a five by five Latin square), a variety of behavioural measures, and adequate controls for such confounding factors as practice effects. The results illustrate the several characteristics of drug-behaviour interactions I have just described.

Dr. Kalant: At what range of blood alcohol level does the reversal of effect on simple reaction time occur?

Dr. Russell: I cannot recall the exact range.

Barbiturates and Behavioural Tests of Disinhibition

Dr. Dews: We have seen these increases in output of behaviour in a variety of situations with common barbiturates. The textbook story is that alcohol and barbiturates are purely 'depressant', and that the increases in output of behaviour that have long been known to occur sometimes following administration of alcohol and barbiturates are not really 'stimulation', but the result of 'depression of inhibition.'

I did an experiment to look into this old story (Dews, 1964). The work was done in pigeons, with behaviour that was maintained by positive reinforcement under a so-called FI (fixed interval) schedule. Suffice it to say here that FI schedules engender a cyclical change in rate of responding, of the general form of a progressive increase in the rate during each cycle, returning to zero at the beginning of the next cycle. During each cycle I periodically superimposed for short periods an additional stimulus that considerably suppressed responding. Thus in a single session I had a range of rates of responding sometimes suppressed by an additional stimulus – inhibited, if you will – and sometimes not. Amobarbital caused large increments of rates of responding. I plotted the results as percentage increases in responding in the presence or absence of the inhibitory stimulus. At the optimum dose level, in the presence of the inhibitory stimulus, the output of behaviour went up about 28-fold on the average, whereas in the absence of the inhibitory stimulus, responding went up less than 2-fold. There was thus an enormous differentiation. It looked as though the inhibited behaviour was especially enhanced—or disinhibited—by the barbiturate.

The schedules of the experiment had been deliberately chosen so that the animals were working at a variety of different rates at different parts of the cycle, both in the presence of the inhibitory stimulus and in its absence, and the rates overlapped. The animals responded faster in the presence of the inhibitory stimulus at some times in the cycle than at other times in the absence of the inhibitory stimulus. I could look, therefore, at the rate increase following amobarbital as a function of the control rate of responding at that time in the cycle. When the results were replotted, taking into account the control rates, the difference between the enhancement by the barbiturate in the presence and absence of the suppressant stimulus disappeared completely. There was absolutely no evidence of a disinhibitory effect of amobarbital.

You find in the literature many dose-effect curves and ratios, such as I have described, comparing responding before and after a dose of drug. Such presentations may be exceedingly misleading, because so many behavioural effects of drugs depend upon absolute levels of the initial behaviour on which the drug effect is superimposed. In the case of the barbiturates, consideration of actual pre-drug levels of responding completely washed out the apparent greater effect on the inhibited behaviour. As far as I am concerned at the moment, there is no rigorous experimental evidence for inhibition of inhibitions as a selective effect of barbiturates.

Dr. Kalant: Is this limited by the maximum output capacity?

Dr. Dews: No. The schedules were chosen to avoid this difficulty (see Dews, 1964).

D. LOCALIZATION OF ACTION IN TERMS OF BIOCHEMICAL MECHANISMS

Dr. Kalant: At this point we have dealt with the inadequacy of localization of drug action in terms of structures; we have also considered a great many of the difficulties of localization of drug actions in functional terms, as defined by effects on specified behaviour patterns, and agreed on the need for a spectrum of behavioural tests. Perhaps we might spend a little time on the subject of biochemical mechanisms of action, to see what we can say about localization in these terms. The drugs that are mentioned in the title of our meeting have been tested with respect to their effects on many different biochemical systems. Have these studies yielded information that is of help in describing or localizing individual differences among the drugs, in terms of their mechanism of action? Or are we faced here, too, with the need to look for still other mechanisms to distinguish among them?

Metabolic Support of a Function and Energy-dissipating Systems

Dr. Wallgren: My impression is that the available biochemical data provide very little basis for predicting specific effects on behaviour. There might be slightly more promise if we think of the addictive drugs but even there I am doubtful. I think the main contribution of biochemistry in this field up to now has been to give some insight into how the drug is acting on the neuronal level in a fairly broad way. We cannot be very specific in relation to various pathways or synaptic complexes or functions, such as inhibitory functions or excitatory functions. I do not think that biochemistry is in a position yet to deal very effectively with that type of question. But some conclusions are permissable concerning the interrelation between the metabolic support of a function and the systems which dissipate energy, mainly from the energy-rich phosphate compounds, in carrying out the specific neuronal function of changes in polarity and transmission or conduction of impulses.

Alcohols, Anesthetics, Cation Stimulation of Respiration and ATPase

Dr. Quastel: There are one or two new observations concerning the biochemical effects of alcohols and anesthetics which might be of interest to people here. There is increasing interest being taken in an enzyme called sodium-potassium-dependent ATPase, which apparently controls the operation of the sodium pump, and which presumably plays an important role in causing cations to move across the neuronal membrane in directions opposite to their concentration gradients.

Dr. Domino: I need a kind of freshman's orientation to this subject. I gather that ATP is important for the sodium-potassium pump, and that ATPase facilitates the breakdown of ATP. What is the connection between the ATPase and ion transport?

Dr. Wallgren: ATPase is a sort of functional system which is involved in the ion transfer phenomenon. Its function is not primarily to split the ATP; its function, though we do not know how it does it, is to move the ions. In this process it uses the energy released by the splitting of ATP; and when the system is activated, it generates ADP at a higher rate by breaking down the ATP more rapidly. In this way it stimulates cellular respiration, because the respiration is controlled through the level of ADP within the mitochondria.

Dr. Titus: The idea is that in the membrane the ATPase provides a microsurface along which translocation of the sodium and potassium occurs, and this is in some way coupled with the breakdown of ATP. It is all highly oriented vectorially, because in the intact cell the potassium has to impinge from the outside and the sodium from the inside, in order to stimulate ATP hydrolysis. The hydrolysis can be blocked by ouabain, but only when the drug is outside the cell; ATP has to hit from the inside. The vectorial character of these stimuli, it seems to me, is good evidence that ATPase is, in fact, the pump.

Dr. Domino: This, I take it, is the crucial point: ATPase is the pump, or at least the ATPase action is coupled with the action of the pump. What is the consequence of this in terms of the membrane potential?

Dr. Titus: This is what maintains the membrane potential.

Dr. Quastel: Let us return to the question of drug effects on this system. Whereas a molecule like ouabain, that blocks this enzyme, also blocks (though less effectively) a cationic and electrical stimulation of brain cortex respiration, a molecule like that of ethanol or the anesthetic tribromoethanol behaves in a different manner. We find that these latter substances, at concentrations that have no effect on this ATPase, block, or partly block, the cationic or electrical stimulation of respiration. The stimulated respiratory activity of the brain cell is highly sensitive to the barbiturates, but quite a number of other molecules can also block this cationic stimulation of respiration. How the alcohols do this is a puzzle to me at the present moment. It would be relatively easy to explain the effect of an anesthetic such as amytal on the ground that it is able to inhibit neuronal mitochondrial respiration at concentrations approximating the anesthetic. But this explanation will not do for the alcohols, because at these concentrations they have no effect upon mitochondrial respiration. This applies also to the highly anesthetic alcohol, tribromethanol (Beer and Quastel, 1958; Quastel, 1965a,b).

My own feeling at the moment is that the alcohols enter into some special relationship with a membrane constituent other than, or additional to, this ATPase, which we know must be concerned with active transport of substances at the neuronal membrane. Moreover, the biochemical effects of the aliphatic alcohols increase with the length of the carbon chain; this is also consistent with their effects clinically. We know nothing at the moment of the details of the relationship between the alcohols and neuronal membrane components. To my mind a knowledge of these components is extremely important for our understanding of the metabolism of the brain cell.

Another molecule we have been interested in is the substance called dimethyl sulphoxide. This interesting substance will act to some extent like a local anesthetic. It is another molecule that can block cationic stimulation of respiration with relatively little effect on the sodium-potassium ouabain-sensitive ATPase. And even an enzyme like phospholipase A, lecithinase, having relatively little effect on the ATPase, has a very large effect on the cationic stimulation of neuronal respiration. This substance, it might be argued, will affect the biosynthesis of ATP. But this does not apply to the alcohols, because at pharmacologically active concentrations their effects on the ATP generating system are relatively small.

One has to think of some special relationship between the alcohols and the components of the neuronal membrane. It may be that a large number of lipophilic substances may act in a manner similar to that of ethanol, by orientation at some type of lipid component that results in a change of the conditions at the membrane, affecting the movements of potassium and sodium ions. This is a vague suggestion, but our present knowledge does not permit anything more specific.

Most of what we might call the soluble-enzyme-controlled reactions that we looked at seem to be undisturbed by concentrations of alcohol which are effective in this way on the stimulated neuronal metabolism. There are a lot of enzyme-controlled reactions which have been investigated, which are quite resistant to the action of the alcohols and barbiturates at anesthetic concentrations. I feel that in order to make headway, we ought to concentrate on just what is happening in this particular system, i.e., the system in which aliphatic alcohols inhibit the cationic or electrical stimulation of brain respiration. I think that this, from the point of view of a neurochemist, would be a very worthwhile piece of work. It must be linked up, of course, with neuronal ATP-ADP interactions, but this is too complicated to discuss here.

Dr. Wallgren: I agree strongly with Dr. Quastel. But I want to emphasize the difficulty of relating all this to the behavioural level. I think it is much easier for us to discuss these things just in relation to function at the neuronal level, and not to go into the final relations to behaviour.

Dr. Dews: When we know in time what the brain does, there is no doubt that it will be found that the physicochemical properties of the constituent cells are competent to subserve this function. But it does not follow from this that the best way to find out what the brain does is to make inferences from the basic physicochemical properties of the cells about how the system as a whole functions.

Ethanol and Active Neuronal Membrane Function

Dr. Wallgren: Ethanol has the very interesting property that it consistently gives a biphasic effect on electrophysiological responses; we first have a lowering of threshold and then an increase. This has been related to the depolarizing effect which has been demonstrated in many systems. We always have this same response in different test systems but, of course, at different concentrations. I think this must be partly, or perhaps wholly, related to a direct membrane action.

Another important point, I think, in relation to ethanol and the other alcohols, is that they apparently act as such by their presence in the system, and not through their metabolites. You can get effects with such metabolites as acetaldehyde, of course, and acetaldehyde potentiates the effects of ethanol. But acetaldehyde is very quickly metabolized, and its effects on various behavioural measures pass off much more rapidly than those of ethanol.

I think that the basic action of ethanol must be on the systems involved in the changes in polarity at the membrane. But somehow producing a decrease in ionic shifts at the membrane, it decreases the requirement for active transport and in this way decreases the rate of regeneration of ATP. There is a rather interesting pattern if we compare different alcohols; as Dr. Quastel has mentioned, there is a regular increase in potency of action with increase in the length of the molecule, in a series of properties. This phenomenon is seen only in an active neural system like the stimulated slice (Lindbohm and Wallgren, 1962) or in impulse conduction in peripheral nerve (Skou, 1958).* There we have very close parallels between the chemical potential of the alcohols in the test system and their biological action. I think this indicates that part of the action at least must be linked with the excitation cycle. But the alcohol effects seem to be very unspecific, and direct effects on transport phenomena also appear to be involved.

*Ed. Note: Rang (1960) has shown a similar relation between potency and chain length in a series of alcohols acting on lung, muscle and other non-neural tissues.

Ethanol and Amino Acid Transport

Dr. Quastel: One very curious fact is that whereas there seems to be no doubt that alcohol must be able to affect cationic movements at the brain cell membrane, it does not seem to affect the active transport of an amino acid like glycine. Ouabain blocks such transport, which is a sodium-dependent process. Alcohol, however, at pharmacologically active concentrations has no effect on it.* Nor has tribromoethanol.

Dr. Domino: Dr. Quastel, do I understand you to say that the transport mechanisms for sodium, potassium and amino acids all get their source of energy from ATP?

Dr. Quastel: Yes, because the amino acid transport is a sodium-dependent process, and ouabain, which blocks the ATPase, also blocks the amino acid transport. This is a well known fact.

Dr. Domino: You say they are linked, and yet by means of drugs, one can dissociate the linkages? Is that not correct? I gathered from your comments that ouabain blocks it, and alcohol does not.

Dr. Quastel: The puzzling thing to me is that with alcohol you can block the effect of electrical and cationic stimulation, and presumably the pump flux. . . . And yet you can do this without affecting amino acid uptake.

Dr. Domino: Then your conclusion would be . . . .?

Dr. Quastel: That there is something else involved, I think, but what it is I do not know.

Dr. Kalant: I suspect this is one conclusion we are all prepared to agree on!

Some work done in our laboratory, by Dr. Israel and others, seems to support the point that Dr. Quastel made, that some other as yet unrecognized interaction between the alcohols and some component of the cell membrane must underlie multiple biochemical actions that we measure. In essence, ethanol and other alcohols inhibit the sodium- and potassium-dependent ATPase activity in the microsomal membrane fraction, from rat brain, guinea pig brain, and eel electric organ (Israel *et al.,* 1965).

*Ed. Note: Recent work by Chang *et al.* (1967), and by Israel *et al.* (1968) indicates that non-lethal concentrations of ethanol cause significant reduction of active transport of the amino acid L-phenylalanine by the intestinal mucosa.

Dr. Domino: From your point of view, Dr. McIlwain, what would you predict would happen to the membrane potential with alcohol, as a result of this action on ATPase?

Dr. McIlwain: We cannot predict it from an action on the adenosinetriphosphatase system alone. To introduce some experimental basis, one is safer in talking about adenosinetriphosphatase systems stimulated by sodium and potassium ions and, as Dr. Titus has pointed out, there are particular orientations to the ions involved in this stimulation. This and a number of auxiliary properties lead one to believe that we do have here a system performing osmotic work. But this is not the only point of interaction of ethanol with systems concerned with movements of sodium and potassium ions across a membrane; ethanol most certainly appears to affect passive movements as well as the active movements that Dr. Kalant has just talked about.

The simplest view regarding an agent which inhibited only the active movements of sodium and potassium ions (which had a specific effect on the adenosinetriphosphatase system, as does ouabain) is that it would diminish membrane potential; and an agent which was acting only on the passive movements triggered by application of a stimulus would maintain membrane potential. There are instances of this in isolated cerebral tissues, which are susceptible to diminution of membrane potential on electrical stimulation. The membrane potential can be maintained by chlorpromazine, phenobarbital and haloperidol, and these compounds can be observed experimentally to diminish the entry of sodium ions and the loss of potassium ions from an isolated piece of tissue. So that there is not a simple answer to what would we expect a compound such as ethanol to do. It certainly would have multiple effects at this purely biochemical level.

Dr. Domino: One can produce depolarization of the neuronal axonal membrane with ethyl alcohol in the same manner as with diethyl ether (Wright, 1947). On the other hand, as you pointed out, barbiturates stabilize the membrane potential, so that at least from the electrophysiological point of view, the actions of alcohol appear to be quite different from those of the barbiturates.

Dr. Quastel: I would like to return to Dr. Kalant's experiments on the effect of ethanol on ATPase activity. What concentration of ethanol?

Dr. Kalant: The same range of concentrations that give substantial inhibition of stimulated respiration and cation transport in slices.

Dr. Quastel: We have not succeeded in obtaining substantial inhibition of membrane ATPase by alcohol at the relevant concentrations.

Dr. Kalant: We have clear results in that respect. The ATPase activity was quite significantly inhibited.

Dr. McIlwain: I believe you found a proportional inhibition, but not 100%.

Dr. Kalant: That is correct.

Dr. Quastel: Would it be 20% inhibition;

Dr. Kalant: No. Ranging from under 20% at the lowest alcohol concentrations which we tested, up to approximately 50% inhibition at 1% ethanol.

Dr. Quastel: Well, I know that at alcohol concentrations that have almost completely blocked electrical or cationic stimulation of respiration, we have not obtained any inhibition of membrane ATPase much above the experimental error of determination, and this is about ± 10% (i.e. about 20% in some cases). Also, Järnefelt (1961) has shown that there is some (but definitely incomplete) inhibition of membrane ATPase at narcotic concentrations of ethanol.

Dr. Kalant: The explanation may lie in the concentration of potassium used. We have found that alcohols and potassium have antagonistic effects on this ATPase activity. Järnefelt did not add any potassium, so that his test system was incomplete. On the other hand, if you add too much potassium you abolish the alcohol effect (Israel *et al.,* 1965, 1966).

Dr. Titus: Has anybody studied the effect of alcohol on cation fluxes in the red cell, where this can be done easily?

Dr. Kalant: Yes, Streeten and Solomon (1954) showed that there was no inhibition of efflux of potassium, but the influx of radioactive potassium was markedly inhibited by ethanol concentrations of less than 0.5%.

Dr. Titus: But was this a pump flux or diffusion flux?

Dr. Kalant: It was active influx of radioactive potassium in red cells which were regaining lost K^+. In our work we did not use electrically stimulated slices, but slices which had lost potassium during a period of precooling, in the case of kidney cortex, or which had lost potassium due to the initial sectioning in the case of the guinea pig brain, or due to a period of anaerobiosis in the case of rat brain slices (Israel *et al.,* 1966). The inhibitory effect of ethanol was measured during the period of active regaining of potassium. Under these conditions, equal concentrations of ethanol gave comparable degrees of inhibition of the potassium uptake and of the isolated ATPase preparation. The inhibition of the ATPase was based on reciprocal antagonism between ethanol and potassium, but not between ethanol and sodium. No concentration of sodium was found which could abolish the alcohol effect; a higher sodium concentration, if anything, enhanced the alcohol effect.

However, these results with the ATPase do not provide us so far with any explanation of the selectivity of alcohol effects upon certain systems or parts of the brain which seem to be the most sensitive. Dr. Israel and I have done a few pilot experiments with the ATPase activity in a calf brain, which provided enough material for separate study of cortex, caudate nucleus, mesencephalic gray matter, etc. But the ATPase from different areas of the brain was apparently inhibited to the same extent by the same concentration of ethanol. We are not drawing any conclusions from this other than that probably we should have tested a variety of different concentrations of ethanol, and probably our separation of sources of gray matter was not as sharp as it might have been. Even so, the possibility remains that there might still have been a difference in the functional importance of the ATPase to these different parts of the brain; so that the same degree of ATPase inhibition by the same concentration of ethanol might have had different functional significance in different areas. Perhaps this type of survey still has to be done, to give a descriptive profile of effects, with all sorts of biochemical processes and other drugs.

To return to the effect on ATPase activity, however, I should like to repeat what I said at the beginning, namely, that I consider our findings to be evidence *for,* and not *against,* Dr. Quastel's view that the primary action of ethanol will probably prove to be a non-specific physical effect on membranes. I do not know the reason for the difference between Dr. Quastel's findings and ours, but I do not think our viewpoint is in any way in conflict with his, because of some further findings that I would like to mention. We have also examined the effects of ethanol on acetylcholine release from brain cortex slices *in*

Ethanol, Acetylcholine Release and Synthesis

vitro, and have tested the effect of ethanol on synthesis in an isolated choline acetylase system from the brain. Here again, we find that ethanol does not inhibit synthesis of acetylcholine, but it does inhibit the release of acetylcholine from the slice at a normal physiological potassium concentration, while it does not inhibit at high potassium concentration (Kalant *et al.,* 1967). This effect is brought about by the same sort of ethanol concentration that inhibits respiration of stimulated brain. Therefore we have no basis for saying that any one of these various actions of alcohol is primary. We consider it more likely that these are different manifestations of some other more basic effect of ethanol upon membrane stability, or configuration, or whatever the proper term is. We might very well find other biochemical processes which are also inhibited by ethanol in the same manner.

Dr. Quastel: Did you say that alcohol stimulates the release of acetylcholine?

Dr. Kalant: No; it inhibits the release.

Dr. Quastel: How is the release effected? What do you use for releasing?

Dr. Kalant: These are brain cortex slices, eserinized and incubated with and without ethanol (Kalant *et al.,* 1967).

Dr. Quastel: You mean you get less free acetylcholine?

Dr. Kalant: Yes, less free acetylcholine with ethanol; but in medium containing 27 millimolar potassium, there is no ethanol effect.

Dr. Hoff: What concentrations of ethanol?

Dr. Kalant: Up to 0.5%.

Dr. Wallgren: Rat tissue?

Dr. Kalant: Yes.

Acetylcholine in Brain Metabolism

Dr. Quastel: Some years ago Hokin and Hokin (1958) showed that acetylcholine can bring about a very marked change in metabolism of isolated brain tissue. It greatly increases the incorporation of radioactive phosphate into phospholipids, but only in the presence of eserine

This is specific for the nervous system. The phenomenon does not operate, apparently, in the other tissues that were examined; neither does it occur in a brain homogenate; it is necessary to have intact tissue. This acetylcholine effect with isolated rat brain can be blocked by ethanol at about 100-150 mM concentration, and by various anesthetics (Brossard and Quastel, 1963). The evidence would seem to indicate that acetylcholine is bringing about its effect by changes in cationic fluxes across the membrane, because precisely similar effects may be obtained by changing cationic concentrations, e.g., those of potassium ions. But whereas the acetylcholine effects are blocked by atropine, and by molecules which presumably compete with acetylcholine for the receptor site, atropine has no inhibitory action on the effects produced by changes in cation concentrations. So one imagines that acetylcholine acts directly at a membrane, bringing about a change in cationic fluxes. We do not understand exactly how this change is brought about, but once it occurs it brings about a series of further changes in brain cell metabolism. What I would like to emphasize is that certain anesthetics, and alcohol too, can block this effect of acetylcholine, presumably by an effect at the membrane itself.*

Ethanol and Ion Conductance

Dr. Wallgren: I think that there is another important effect of ethanol shown by the findings of two independent groups (Moore, Ulbricht and Takata, 1964; Armstrong and Binstock, 1964) concerning the changes in ion conductance during action potentials in squid axon. Armstrong and Binstock used an ordinary voltage-clamp combined with intraaxonal recording, whereas Moore *et al.* used a sucrose-gap voltage-clamp technique. The Armstrong and Binstock preparation was more sensitive and showed a large inhibitory effect of ethanol on the increase of sodium conductance in the rising phase of the action potential, and a much smaller effect on the subsequent increase of potassium conductance. The preparation used by Moore *et al.* was less sensitive, but their conclusions were basically similar.

In relation to stimulated cerebral cortex slices, as we have been using them, I would predict that one of the effects of ethanol will be a slowing of the entry of sodium and the efflux of potassium which occur on stimulation, and which trigger the increase in transport of sodium out of the cells and of potassium back into the cells. We have actually started the measurements but do not have good data yet. We all seem to agree that it is important to pursue the whole question of the effects of ethanol and several of these other substances on the kinetics of ionic movements, and the mechanisms underlying ionic movements across the cell membranes. Many approaches are possible using model systems or various neural preparations. Such studies can be related to the effects on the various neurohumoral transmitter substances which presumably act at this level and thus have the possibility of interaction with the drugs.

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*Ed. Note: See the later work by Gage and Quastel (1965) concerning the acute effects of ethanol on the release and action of acetylcholine at the neuromuscular junction.

Dr. McIlwain: It is certainly attractive to see effects of ethanol on active and passive sodium and potassium movements. Some data are already available in relation to the other substances that we have been discussing. Phenobarbitone does affect such movements as also does chlorpromazine, inhibiting the entry of sodium and therefore the metabolic consequences of that: the increased ATPase activity, and increased respiration.

Dr. Quastel: They also have effects on the ATP levels, do they not?

Dr. McIlwain: Well, I rather doubt that in the case of chlorpromazine. My impression was, until you mentioned this observation, that the concentrations of chlorpromazine necessary to affect this system were much beyond those existing *in situ* during the action of the therapeutic dose.

Dr. Quastel: Do you mean the levels were measured?

Dr. McIlwain: The cerebral concentration was measured after the drug had been taken.

Dr. Titus: It seems to me you have to differentiate between effect on this ATPase which, as I see it, would influence the ability of the cell to maintain its state of excitability, and effects on depolarization, which may be an entirely different phenomenon. This would make the differentiation between these drugs a little more sensible.

Dr. Kalant: Is this perhaps an impossible differentiation at present? Since one cannot fully solubilize the ATPase and test for drug inhibition in a homogeneous system, one must in fact use fragments of membrane as the ATPase preparation. Any drug effect on it may well be a membrane effect of the same type as that underlying depolarization.

Dr. Titus: True. But in the intact cell—and here I am thinking primarily of the work of Tosteson and Hoffman (1960) with red cells—you can differentiate between passive fluxes, which are relatively large in these cells, and pump fluxes; and you can operate on these independently. With ouabain, for example, you can specifically inhibit an energy-dependent pump flux. Drugs like vasopressin, in contrast, do nothing to the relationship between pumping and the energy supply, but change the physical structure of the membrane in some way so that the diffusion fluxes change. It may very well be that operations on the transport system are involved in the actions of alcohol, chlorpromazine and so on. It seems to me that one of the most interesting things that has emerged from several years of biochemical investigation is that whenever any of these drugs seem to have an effect on a metabolic system in brain, it is always mediated through an enzyme. Only those metabolic changes which result from potassium chloride stimulation or from electrical stimulation of the preparation are affected by the drugs. Some change in the relationship between membrane structure and the intracellular enzymes seems to be the key to the situation here.

Dr. McIlwain: I most certainly agree. The adenosinetriphosphatase may not have been solubilized entirely, but it has been at least obtained in a micellar form (Swanson *et al.*, 1964; Tanaka and Strickland, 1965) which is very remote from that of an organized membrane, and if substances affect it in this form, then I think one is dissociated to a certain extent from properties of, say, surface pressure, which must operate very differently when the enzyme has been removed from its normal environment. It may be put into another organized environment but at least it is different from the lipid constituents of the microsome fragments.

Dr. Himwich: Ouabain is extremely effective in the isolated tissue.

Dr. McIlwain: Yes. And I would think that with ouabain, one does have an action at an enzyme active centre.

Dr. Kalant: Have any of the drugs that we are discussing here been tested with the micellar preparation of ATPase?

Dr. Titus: What about chlorpromazine? Did somebody test this?

Dr. Quastel: Chlorpromazine at quite high concentration knocks out ATPase activity.

Dr. Titus: But you need to use quite a large amount?

Dr. Quastel: Oh, yes; very large.

Dr. Titus: Well, we have tried desmethylimipramine, which is not a cellular depressant, and it has no activity whatsoever on ATPase.

Dr. Quastel: But chlorpromazine is able to inactivate almost any enzyme if used in high enough concentration. It reacts with all sorts of protein just as methylene blue does.

Dr. Kalant: If one thinks that the same ATPase activity has also been blocked by diphenhydramine, by ouabain and by other apparently unrelated drugs (Judah and Ahmed, 1964) such as chlorpromazine in a concentration which is not too far removed from that prevailing *in vivo* with therapeutic doses, it makes one very reluctant to say that this effect on ATPase can be a primary action. As Dr. Quastel said earlier, we must look for some basic physical interaction between some constituent of the cell membrane and the members of this rather large group of drugs (which are otherwise chemically quite different from one another), which alters the cell membrane in some way that results in a series of different functional impairments. Perhaps we are not yet in a position to relate any of these biochemical impairments to the neurophysiological and behavioural changes that we consider characteristic of the various drugs.

Ethanol, Depolarization and End-Plate Transmission

Dr. Wallgren: In relation to what Dr. Domino said (p. 157) about the effect of ethanol as a depolarizing agent, I might say that there is a little evidence for an increase in the passive permeability to ions in some systems. For instance Hurwitz, Battle and Weiss (1962) showed that with smooth muscle, ethanol increased permeability to potassium; but on the other hand, it largely blocked the increase in potassium permeability which was caused by acetylcholine, so that there was some interaction between acetylcholine and ethanol in this respect. But ethanol differs from other alcohols in its properties, because the higher analogues do not depolarize. The exact relationship among a series of alcohols depends on the preparation you use. Posternak and Mangold (1949) used frog sciatic nerve and found that ethanol was depolarizing, and so also was propyl alcohol, but to a lesser extent. Butanol had no effect upon the resting potential, whereas the higher alcohols were hyperpolarizing agents. But all of these blocked impulse conduction at about the same chemical potential (thermodynamic activity), and I think this is related to a specific effect on the mechanisms underlying the action potential, which presumably is another aspect of the membrane effect. Therefore we might say that ethanol actually is a membrane stabilizer in the sense that it interferes with the generation of the action potential; but it might also slightly alter the properties of the resting membrane in the same direction as do the labilizing agents.* Alcohols generally seem to be rather similar in blocking the impulse conduction, but different in their effects on the resting membrane.

Dr. Kalant: Are you suggesting, perhaps, Dr. Wallgren, that there are two different processes with slightly different dose-response or concentration-response curves? The result of summation of the two effects, with the lower alcohols, is a biphasic curve, and with the higher alcohols, a monophasic depression?

Dr. Wallgren: Yes, it appears so. I would think that the essential aspect, anyway, is the effect on the mechanisms underlying the action potential, but this should be explored more thoroughly.

Dr. Domino: If I understand correctly, you are saying that ethanol produces some depolarization, and this slight depolarization has a facilitating effect. At much greater concentrations the depolarization would go all the way?

Dr. Wallgren: Yes, that is actually what happens. And how to interpret this in terms of stabilizers or labilizers, I really do not know. I am confused about it myself because in the case of ethanol there is this biphasic response, whereas with the higher alcohols there is not (Posternak and Mangold, 1949).

Dr. Kalant: A few years ago, Basmajian and his collaborators (Low *et al.,* 1962) tested the effect of ethanol on ulnar nerve conduction in the intact human subject. They stimulated at the elbow and at the wrist, and recorded action potentials in the hypothenar muscles. The difference in the respective response times was taken as the impulse conduction time from elbow to wrist. Using two different doses of alcohol – 1½ and 4½ ounces of whiskey – they found no change in velocity of conduction between the elbow and the wrist. But there was a slight shortening of the time between stimulation of the ulnar nerve at the wrist and detection of the hypothenar muscle potential, indicating that end-plate transmission had been slightly speeded by the alcohol. Does this not appear to contradict the idea that there is an important basic membrane-stabilizing effect in peripheral action?

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*Ed.Note: Later work by Seeman (1966) indicates that all lower alcohols exert a membrane-stabilizing effect on erythrocytes, which reduces passive movement of ions across the membranes and so protects against osmotic rupture.

Dr. Wallgren: I would not say so really, because in this preparation, with the doses used, we are apparently within the level of the biphasic reaction related to a depolarizing effect. I do not see how we could really be sure that this is not just a first early manifestation of a basically similar mechanism which, when you increase the concentration of ethanol, finally results in blocking impulse conduction and transmission. How can we be sure that this is qualitatively different from what happens at the higher concentration?

Dr. Brazier: Do you really conceive of the finding of Low *et al.* (1962) having all been the effect of ethanol in the periphery?

Dr. Kalant: No. On the contrary, I feel that the effect is not primarily on the peripheral neuron, but I am not sure whether there is good evidence of central facilitation or inhibition of transmission at the neuromuscular junction. If there is, I would think it more conceivable that the shortening of the time of transmission at the myoneural junction was a central effect of ethanol rather than a peripheral one.

Dr. Wallgren: But with an isolated peripheral preparation you get the same kind of response within the same concentration range. It might well have been a local effect.

Dr. Domino: The Russian physiologist, Orbeli, found many years ago that the sympathetic nerves facilitate neuromuscular transmission. It is possible to modify neuromuscular transmission indirectly via an action on the sympathetic nervous system either centrally or peripherally. The point that Dr. Wallgren makes is that you can show the same phenomenon in the isolated system, which certainly rules out any Orbeli phenomenon. Furthermore, it would suggest that in this case the action of ethyl alcohol, if it were through an Orbeli phenomenon, would be to increase the sympathetic activity. Does it?

Dr. Wallgren: Well, there is some evidence that it does, so I think we must also consider that interpretation. But there is positive evidence for isolated peripheral preparations going through the same phases of ethanol effects, and actually Low *et al.* did not test higher doses. The blood alcohol levels of their subjects varied from about 20 to 100 mg/100 ml. They might well have been within the range in which you see this facilitation. I do not think we have any valid basis for deciding between the various alternative explanations for their findings, because the experiments on the basis of which we could select the correct alternatives have not yet been performed.*

Barbiturates and ATPase

Dr. Domino: From the point of view of the sodium-potassium-stimulated ATPase, is there any evidence that the depressant and the convulsant barbiturates differ in their actions? I am thinking, for example, of pentobarbital as opposed to 1, 3-dimethyl-5-butyl-5-ethylbarbituric acid.

Dr. McIlwain: I am not aware of any.

Dr. Domino: Brody and Bain (1954) showed years ago that both of these compounds cause uncoupling of oxidative phosphorylation. We have shown that the convulsant barbiturates do have depressant properties. I wonder whether any more recent biochemical differentiation or similarity has been shown.

Dr. McIlwain: I am not aware of it in the case of these compounds. There have, however, been differences noted between thiobarbiturates and other barbiturates in terms of uncoupling potency. In fact, Aldridge would say that barbiturates other than thiobarbiturates are definitely not uncoupling agents, and that one should look very carefully at the experimental evidence that claims that they are uncoupling agents. I think some of Brody and Bain's observations do show a clear uncoupling in the case of thiobarbiturates, and this had been extrapolated to other barbiturates where it may not have been by any means clearcut. But I am not aware of differences between the convulsant and other barbiturates at that type of biochemical level.

Dr. Quastel: The thiobarbiturates definitely uncouple. But the oxybarbiturates prevent phosphorylation and the oxygen consumption also diminishes so that the P/O ratio is constant. The processes are not really uncoupled, they are depressed.

*Ed.Note: The shortening of residual latency on ulnar nerve stimulation in man, following small doses of ethanol, was later found to be secondary to rise in temperature of the arm as a consequence of ethanol-induced vasodilatation (Peiris *et al.*, 1966).

Dr. Dews: In situations in which pentobarbital causes an increase in the output of behaviour, 1,3-dimethyl-5-butyl-5-ethylbarbituric acid causes a decrease.

Dr. Domino: You have observed this?

Dr. Dews: Not I, but Waller and Morse (1963) in our laboratory.

Dr. Domino: But it has been reported that they go in opposite directions?

Dr. Dews: The 'depressant' barbiturate caused an increase and the 'stimulant' barbiturate a decrease in responding of pigeons under a fixed ratio schedule of reinforcement. If one measured output of 'convulsant behaviour', presumably, the drugs would be the other way round. Once again we see that the terms 'stimulant' and 'depressant' are worse than useless unless the behaviour under study is simultaneously specified.

Sequential Phenomena in CNS

I would like to go back to a question that came up a little earlier (p.166), in relation to the effects of ethanol on neuromuscular transmission and on central synapses. The type of action of a drug does not necessarily have to be different in the periphery and in the central nervous system. Ethanol may not have any bigger effect at any single CNS synapse than at a peripheral synapse but in the CNS you may have sequential chains of synapses. Then the effect of alcohol would be like compound interest. If you get 5% decrement at a synapse, and another 5% at the next step in the chain, another 5% at the next, and so on, when you have been through a couple of hundred synapses the total effect in the chain is very large, even though at each individual step it might be quite small. If such a process were taking place, a gross change in behaviour might result from an undetectable change at any single synapse involved. Such a gloomy view of the potential contributions of microneuropharmacology may be quite unjustified, however; the pharmacologically important pathways in the brain may be characteristically multiple parallel paths rather than sequential series paths.

Dr. Kalant: If your initial suggestion were correct, one would expect to find the opposite to what Dr. Domino mentioned yesterday about the effects of ethanol on polysynaptic and monosynaptic pathways. He referred to Kolmodin's report that there was no appreciable difference between them, yet one might expect that there should have been a fairly substantial difference. Certainly, if the effect of ethanol on acetylcholine release which is observed in the stellate ganglion and in brain cortex slices also prevailed at peripheral synapses, one should also have seen a difference between the mono- and polysynaptic pathways.

Dr. Domino: In our own research with Kissel (1956), we found a very minor selectivity of ethanol, in affecting mono- and polysynaptic reflexes differentially, but certainly the best data are Kolmodin's and he has concluded that there is no selectivity.*

E. ADAPTATION IN THE CNS

Dr. Domino: So far we have been talking mainly about acute effects of these drugs. Let us return to the hypothesis I presented in my paper regarding barbiturate addiction, based on Eccles' conclusion that these compounds prolong presynaptic inhibition (see p.46).

Enzymes, their possible Induction or Inhibition

How could you test such an hypothesis? If a mechanism of enzyme induction is involved, then by using substances such as ethionine which will inhibit enzyme induction one ought to reduce the amount of physical dependence on barbiturates. This interesting prediction could be tested not only with respect to the barbiturates, but to ethyl alcohol, to narcotic analgesics, and to other compounds that produce a physical dependence.** This kind of experiment is a direct consequence of the electrophysiologic data, which tell us the direction we ought to move in for future biochemical studies. On the other hand, if the drug interferes with an enzyme that destroys a transmitter, less of the transmitter is necessary for the organism to carry on its given function. In the case of presynaptic inhibition, the nature of the transmitter substance is unknown. A lot of fundamental research on transmitter substances in the central system is required and agencies interested in drug addiction would do well to support basic research into questions such as this. Total levels and turnover of acetylcholine, serotonin, catecholamines and so on, need to be examined in relation to the problem of physical dependence. Some of this has already been done and has been, to a certain extent,

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* See footnote on p.40.

**Such an approach has been used by Way *et al.* (1968) in their studies of the effects of cycloheximide and p-chlorophenylalanine on the development of tolerance to and physical dependence on morphine.

Changes in Transmitter Effects or Metabolism

a blind lead. Nevertheless, the hypothesis is still a good one. Other potential transmitter agents also need to be examined in this framework. It further emphasizes the fact that neuropharmacologists who have hitherto concentrated on an electrophysiological approach must become more involved with biochemical studies. There is a great need for further interdisciplinary research, or cross-communication and fertilization of ideas among many different specialties.

Membrane Changes

Dr. Wallgren: To understand what happens in the development of tolerance and in the appearance of the withdrawal symptoms, we have to know the acute effect of the drug. It is the acute effect that in the first place induces an adaptive change that results in the restoration of efficient function when the drug continues to be present. We might picture this adaptation perhaps as involving, as Dr. Domino suggested, a change in transmitter effects or in transmitter metabolism. Also, in relation to long-term effects, we might think of an adaptive process in the membrane constituents, an alteration of structure or organization perhaps, a partial reorganization or synthesis of some different components, or something like that, which compensates for the continuous presence of the interfering drug.

Dr. Titus: One of the difficulties faced by biochemists in a group like this is the fact that the biochemist necessarily works with very simplified systems in reactions between two molecules, and must always try to sift through the information obtained from very highly integrated systems to find some component of those systems upon which he can operate experimentally.

But then comes the necessity of deciding where the biochemist can make some contribution to a little larger area. Here I would like to speculate and suggest experiments that are, at least in principle, possible. This has to do with some comments which Dr. Russell made in the discussion following his paper. I do not think we will ever be able to find one of his drive stimuli, upon which we could operate biochemically. But if, as in morphine addiction, the development of physical dependence and its attendant discomfort plays a big role in the system, it is possible that a biochemist could derive some information about the mechanism of physical dependence. It may very well be that either morphine itself, or the change in state of, let us say, the post-synaptic membrane upon which morphine may act, might have inductive consequences tending to change the biochemical nature of this membrane, so that it becomes hyperexcitable. This could conceivably be by the synthesis of new proteins, possibly enzymes, as

Dr. Domino has suggested. If this is an inductive change, involving protein synthesis, then it is testable with the sort of techniques that have been used to study the induction of the drug-metabolizing enzymes in liver, such as prevention of protein synthesis by agents like puromycin, or prevention of the synthesis of messenger RNA with things like actinomycin D. This approach to the study of induction of drug-metabolizing enzymes has been extensively reviewed by Conney (1965).

This would have been difficult to conceive of a few years ago, because it was generally accepted for some time that messenger RNA, which takes the information from DNA and governs the synthesis of the protein, turns over very rapidly. But now it appears that in mammalian systems there are quite frequently messenger RNA's that turn over relatively slowly (Marks *et al.*, 1962; Garren *et al.*, 1964; Revel and Hiatt, 1964; Bloom *et al.*, 1965), the time being such as to allow for the development of drug dependence, which, I think, takes 24 to 36 hours or several days. It could be that experiments of this sort would reveal whether or not there is in fact an inductive component in the development of physical dependence. This is probably one of the very few areas in which the biochemist can begin to get into the kinds of phenomenon that concern the behavioural psychologist. In fact, one stimulating result of this conference is that I would like to go home and try this sort of thing; I do not believe this kind of approach has been used yet.

Dr. Russell: In order to do this, what would be the first step: finding out whether the drug itself did induce changes in protein synthesis?

Dr. Titus: I do not know how to differentiate between the drug itself as an inducer and some drug-effected change in the state of the membrane as an inducer. If you use the experience in the liver enzymes as the model -- and this is admittedly a poor model because these inductive effects occur only in liver – it would seem that you can get induction of microsomal drug-metabolizing enzymes by relatively simple changes in structure. Remmer (1962) has shown that diethyl ether or nitrous oxide can induce these microsomal enzymes. Some of the inductions can be blocked in the intact animal by giving puromycin, or actinomycin D. Both of these are unfortunately somewhat toxic, actinomycin D particularly, but they can be used *in vivo* and it might be worth trying.

Dr. Russell: Both of those agents have been used in studies on the effects of learning (Flexner *et al.*, 1963; Barondes and Jarvik, 1964).

Dr. Titus: Flexner and his associates (1962) did this.

Dr. Russell: Yes. Both with actinomydin D and puromycin, the reports have indicated that the retention of learned behaviour patterns, involving the storing of information, is differentially affected after administration of these two agents.*

Dr. Titus: There may be some complications in these experiments. The whole thing arose in the beginning from experiments with messenger RNA in E. coli, in which it plays a big role and does turn over very rapidly. With microorganisms, it is sometimes difficult to demonstrate an effect with actinomycin D because of permeability problems. Changes in membrane properties, caused by chelating agents such as EDTA, can apparently make the actinomycin effective (Leive, 1965). I anticipate some experimental difficulties which may arise in the use of these drugs, but even so, I think it would be well worth a try.

Dr. Domino: I prefer ethionine in contrast to these other agents, because of the problem of the blood brain barrier. Certainly puromycin and antinomycin D would have to be applied directly to the surface of the brain as in the case of Flexner's work involving the mouse, and more recently Agranoff's work in the fish (see Agranoff *et al.,* 1967). If one were to use these agents to test the assumption that physical dependence to drugs is due primarily to an action in the central nervous system, then one would have to use the intraventricular or intracerebral route of administration. Then you get into the problem of the direct pharmacological effects of these two substances which, I believe, in large doses produce convulsions. In any event, it might be interesting, for example, to take one series of animals and give these drugs parenterally, with the idea that not much is getting into the brain, and see how the picture of physical dependence changes, and then to do another set of experiments where the same materials would be given intraventricularly. Perhaps in this way we can get at the central and peripheral disturbances in drug-induced physical dependence.

Dr. Russell: Or given intracisternally, in the way Dingman and Sporn (1961) did with 8-azaguanine.

*Ed. Note: Cohen and Barondes (1967) report that the puromycin effect on memory may be due to occult seizures rather than to inhibition of protein synthesis.

Dr. Himwich: Dr. Titus, when you give one dose of phenothiazine, you get a certain kind of result, and if you repeat it next week you get the same result, and also if you repeat it four weeks after that. But if you give a phenothiazine day after day, changes in response are observed. For example, EEG arousal can be blocked by the action of chlorpromazine when given acutely, but when chlorpromazine is administered chronically there may be even spontaneous EEG arousals (Himwich, 1965). Does this fit into your concept of drug-induced changes?

Dr. Titus: I do not know.

Dr. Wallgren: I suppose that Dr. Titus' idea is that probably you should be able to prevent this by having administered puromycin or actinomycin D.

Dr. Titus: In the fish, you assume that the learning process involves enzyme induction because drugs which interfere with protein synthesis prevent it.

Dr. Wallgren: Yes; and you are now implying that if the addictive process involves synthesis of new forms of protein, then we should be able to prevent addiction by preventing protein synthesis.

Dr. Titus: It might be a response by, let us say, a post-synaptic membrane that is not receiving the stimuli that it normally does; it might have some mechanism enabling it to synthesize more receptor sites, under this condition.*

Dr. Kalant: I would like to ask an uninformed question at this point. My understanding of the adult nervous system is that cell duplication does not take place. Is there good evidence for modification of enzyme spectrum in neurons of the adult nervous system as a result of environmental factors, and evidence that such modifications can be prevented by agents which interfere with protein synthesis?

Dr. McIlwain: There certainly is protein synthesis in progress in the brain. I do not recall good evidence for a difference in balance of protein constituents synthesized under the influence of drugs.

*Ed. Note: See Collier's hypothesis (1965).

Dr. Domino: What about Hydén's work using malononitrile and other compounds of this type which stimulate protein synthesis?

Dr. McIlwain: That is a very toxic compound and I am not certain of the physiological status of the results.

Dr. Titus: But one of the compounds that Hydén used was imipramine. According to the report I read (Hydén, 1962), imipramine caused a change in the base ratio of RNA, first in the glia and then in neuronal nuclear RNA. The base ratios in the newly synthesized RNA would suggest that it was messenger RNA that was changing, which, if true, was a very exciting result. But I have not read the original paper, and I have no really critical view of it.

Dr. Himwich: With regard to Dr. Domino's concept on addiction: the barbiturates have an affinity for the spinal cord, is that correct?

Dr. Domino: They can certainly act there to depress polysynaptic pathways when given in low doses, and monosynaptic pathways in larger doses. In addition, they prolong presynaptic inhibition.

Dr. Brazier: Eccles' group used, I think, only pentobarbital in their studies. One has to show that presynaptic inhibition takes place elsewhere than in the spinal cord, which is a difficult task, but it would be interesting in their particular preparation to do it with tagged barbiturates.

Dr. Kalant: Dr. Domino, your model may or may not be pertinent to higher levels of the central nervous system. Should we not ask whether the concentrations which are required to produce such effects on the spinal cord are relevant to the *in vivo* effects on the whole animal? I would like to ask, also, whether this is a point of differentiation from ethanol. Yesterday we were talking about the finding that ethanol inhibits axonal conduction at concentrations lower than those required to affect synaptic transmission.

Dr. Domino: To my knowledge, the effects of ethanol on presynaptic inhibition have not been studied.* But chloralose has the same effect as the barbiturates on presynaptic inhibition, and also has important

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*Ed. Note: Enhancement by ethanol of presynaptic inhibition in the unanesthetized spinal cat was later reported by Miyahara *et al.* (1966).

actions on postsynaptic inhibitions, apparently not possessed by barbiturates. The spectrum of pharmacologic actions of chloralose is thus rather different from that of the barbiturates. I do not know whether there is such a phenomenon (or was in the old days when chloralose was used in man) as chloralose addiction and physical dependence.

Dr. Brazier: Chloral hydrate addiction was surely seen!

Drugs and Presynaptic Inhibition

Dr. Hoff: Yes. It might be worth while to see whether prolongation of presynaptic inhibition has specific characteristics at different levels of the nervous system. This might lead to a sounder approach to the studies of the systematic neurophysiologist, in selecting those areas for stimulation and production of, say, the autonomic end-effects, to see whether he can predict which stimulation loci are likely to be most affected by the drug.

Acute Neuronal Adaptation

Dr. Domino: Alcohol and certain other drugs produce the phenomenon of neuronal adaptation. An organism, be it an animal or man, may initially show some evidence of EEG effects following administration of ethanol; yet even though the blood level of alcohol is maintained, there is then a tendency for the organism to return towards a normal state, in terms of EEG signs as well as gross behaviour. Although the blood level is maintained, in a span of minutes or hours the neuronal tissue has already adapted to it. One can argue that measuring blood levels of alcohol is not the crucial thing, and that we should measure brain levels of alcohol.

Dr. Wallgren: It has been done, and it is well known that brain and blood levels correlate very well (Elbel and Schleyer, 1956). It was studied in relation to tolerance, by Newman and Lehman (1938) long ago.

Dr. Titus: You have to differentiate between acute tolerance that we are talking about here, and other kinds of tolerance. The sort of thing you are talking about with alcohol also turns up with some other drugs; I think carisoprodol is one. You can show this by giving rats a dose of about 200 mg which puts them to sleep, and then measuring blood levels of the drug when they wake up. If you increase the dose from 200 mg to 300 mg, the animal will wake up at a much higher blood level of the drug. I think the comparative figures are about 92 and 165, which is a striking difference. This acute tolerance sets in within minutes after the dose and is dependent on the size of the dose that you give. It is completely independent of any development of tolerance due to changes in drug metabolism, which come on considerably later.

Dr. Dews: This is true of barbiturates too, isn't it?

Dr. Kalant: In respect to ethanol this has been known for a long time as the Mellanby effect (Mellanby, 1919). It must be a general phenomenon, I am sure, with central nervous system depressants.

Dr. Domino: In the case of reserpine, brain levels of serotonin can be fairly low during the recovery phase even though the animal's behaviou is fairly normal.

Dr. Himwich: That is another phenomenon. An important factor involved in these differences in serotonin is the discrepancy between the relatively large amounts of stored serotonin and the very small amounts in intracellular granules which, nevertheless, are adequate for the function of synaptic transmission (Carlsson, 1964).

Dr. Russell: May I ask a general question at this point? In searching for a neurochemical substrate to correlate with the effect of drugs on behaviour, is it right to say that we are really looking for a series of neurochemical events, all of which may be necessary for the behavioural effects observed, and no one of which may be sufficient in itself? In other words, to look for a single specific neurochemical correlate of behaviour might be misdirecting our attention. Is this a significant possibility?

Dr. McIlwain: This brings me back to a comment made before on biochemistry and behaviour. There is growing up a phraseology in this subject in which we nearly always use the word 'correlation', – 'biochemical correlates of behaviour.' Well, unfortunately, matters often remain at the level of correlation, and it has not been possible to visualize more mechanistic interactions. Biochemical correlates of behaviour take one only a very short distance. What we may see, perhaps during the course of a generation, is a radically different appreciation of just how the brain does perform adaptive changes, and we may see them in a way that does not take models at all from microorganisms or other mammalian organs, so that biochemical correlates may not give much explanation of the nature of these adaptations.

Dr. Russell: We could get into a lengthy discussion of the logic of scientific method, and particularly of cause and effect relationships, which are difficult to define. In talking about cause and effect we have to agree on what we will call 'cause' and what we will call 'effect.' Frequently the distinction is based upon the temporal order of events, in the sense that an event which follows another is considered the consequence or effect of the latter. But it must be established that the two are actually involved in the same chain or sequence of events. Even if we are some day able to understand in much greater detail the mechanisms that are involved under the circumstances that Dr. McIlwain has discussed, I think that we may still choose to speak of 'correlates' of the behaviour rather than cause-effect relations, meaning by 'correlates' the events that vary concomitantly.

Dr. McIlwain: To return to the microorganism: practically all aspects of its behaviour are in fact biochemical; but in mammals one cannot very often see logical connections of biochemical events with overt behaviour. The process of biological evolution appears to have separated these two, so that very much less of the overt behaviour of a higher organism is expressed biochemically, though it certainly needs a basis in biochemical processes and mechanisms. But it has proceeded so that the manifestations of behaviour are no longer primarily biochemical. My comment was not about an abuse of the word 'correlation', or 'concomitant variation', but just an observation that this may be all that remains in the subject when you reach the mammal, and especially when you reach the functions of the brain.

Dr. Himwich: The ability of the brain to achieve functional recovery after an injury was first emphasized by Hughlings Jackson. Later we began to think of an explanation in terms of 'reverberating circuits.' It seems that some elements of these reverberating circuits remain and continue functioning to some degree despite brain damage in other areas.

Plasticity of Brain Function

Dr. Dews: One of the extraordinary things about the animal brain is its ability to compensate for all sorts of insults. Let us take some specific examples. As you know, there are regions that, when stimulated, cause eating; when these regions are coagulated, the animals will stop eating altogether. If left without forced feeding for a period, they just die. But if they are kept alive with stomach tubing, after a relatively short time these rats will take milk chocolate as a first form of nutrition; and over a period of weeks they can be gradually weaned back until they will maintain themselves on ordinary food. In some wa this lesion, which is initially lethal, can be compensated for.

I believe the same sort of thing is true in regard to regions in the midbrain reticular formation, coagulation of which causes the animal to go into a coma. If you manage to keep it alive for a few weeks, then it will 'come awake.' The same is true with the lesions that are made in thalamic areas to block pain pathways in patients with terminal cancer in the head and neck region. You can make a lesion in the thalamus and alleviate the pain. If the pain comes back, you make a bigger lesion and aga you get relief for two or three weeks until the pain comes back yet agai You can go on until you have coagulated a huge volume of the thalamus producing a lesion that would initially be completely incapacitating to the subject. So the brain must have an extraordinary ability to compensate for chronically imposed changes.

Now perhaps the tolerance to morphine is an example of this sort of compensation. It may be that the particular areas that are affected by morphine are peculiarly able to be compensated for, and this enables the high degree of tolerance to develop. If you look at the problem from this broader context, it becomes a little more difficult to move so quickl I think, to specific problems of protein synthesis, such as we were discus ing earlier.

Dr. McIlwain: I certainly second that. Yes, it strikes one that the brain par excellence has made a great specialty of adaptation and adjustment and it would be purely an assumption to think that it has made this after the pattern in which the liver can make adjustments of enzyme activity. In fact, I think the balance of general biological feeling would be against this.

Dr. Titus: I would hardly propose the liver as a model for the brain! Th liver happens to provide the only instance I know of drugs causing induction of enzymes.

Dr. McIlwain: It strikes one that the nervous system may have developed in a radically different way from this – away from all sorts of properties found in unicellular organisms and away from properties of an organ suc as the liver. There are so many aspects of its behavioural activities which are no longer strictly at a biochemical level, nor do they have the same direct biochemical interpretation.

Dr. Wallgren: I would like to recall the plasticity of brain function cited by Dr. Dews (p.177), and its ability to compensate for the anatomical removal of parts of it. Is it known whether increase in tolerance to the effects of a drug, manifested as more efficient functioning at the same level of the drug in the brain, is associated with the incorporation of new pathways which were not functioning before drug-taking started? Is tolerance a manifestation of such plasticity in brain functions? This has not been investigated in relation to ethanol.

Dr. Kalant: Do you mean new neuronal pathways or new metabolic pathways?

Dr. Wallgren: Neuronal pathways.

Dr. Russell: I know of no studies relating to changes occurring during the development of tolerance.

Dr. Dews: It seems to me that the problem is that you can trace some of the input of information and its processing as far as cells in cortex, and you can trace back outcoming signals as far as the cortex. But there are still pathways between which are not defined. There are multitudinous interactions within the cortex, and between the cortex and the basal ganglia, the cerebellum, and other parts of the brain—impulses shuttling backwards and forwards all over the place. The gap between how far you can trace information in, and from how far back you can trace instructions coming out, is still very big and most of the compensatory changes presumably take place in the mysterious pathways of the gap. We do not even know that the gap is bridged by pathways of the same nature as, say, the visual pathways from eye to occipital cortex.

Dependence in Structures Other Than CNS

Dr. Domino: I think we all agree on the importance of the ability of the brain to adapt to change, and the possible role of this adaptation in the addiction process. But I keep coming back to the question of whether it is simply because the brain is so likely to show evidence of change that we consider it the most important site of drug-induced physical dependence. The research by Corssen and Skora (1964), for example, on the effects of morphine on human cancer cells in tissue culture, certainly does raise the question: is this a property common to all kinds of cells? With any drug that produces physical dependence, are we really also getting physical dependence of the liver, kidney and muscle? With regard to morphine in particular, there is an old study of Shideman and Seevers (1942), showing that skeletal muscle gives some evidence of morphine withdrawal effects in terms of change in oxygen consumption.* I do not know whether this has been confirmed or not. I do know that Seevers himself feels that this phenomenon is not important to the overall problem – that really morphine withdrawal symptoms are a manifestation of disturbance of the brain and not of peripheral tissues. But I would like to see more evidence in that regard. I think it is important from a biochemical point of view, because if there is harmony in nature and if all cells operate by more or less the same fundamental mechanisms, then perhaps it would be possible to use models of other tissues in studying biochemical reactions of drugs, that might be ultimately applicable to the brain.

Dr. Dews: The possibilities you mention are not exclusive, of course. There is no reason why both these could not be operative.

Dr. Wallgren: Seevers and Deneau, in their review (1963), emphasized strongly that physical dependence is related to the central nervous system. They think there is no conclusive evidence that so-called dependence phenomena in other tissues are really equivalent to those in the nervous system.

Dr. Kalant: What about the work of Sasaki (1938), and the publication that Dr. Domino mentioned by Corssen and Skora (1964), on tolerance and dependence on morphine in tissue culture?

Dr. Wallgren: Yes. I think there is some evidence, really. Seevers and Deneau (1963) quoted it in their review, and they interpreted the evidence which was available in relation to the topic they were discussing, namely tolerance and dependence to drugs acting on the central nervous system. Whether something similar occurs in other tissues, we do not really know . . . . quite possibly it does. I am sure of the central nervous component in connection with ethanol; we have very clear withdrawal phenomena; we have also another kind of more diffuse dependence that probably is due to peripheral metabolic alterations, which relate to the role of ethanol as a food.

It would be very interesting to have a preparation in which you can definitely show a change within specific cells during the process of acquisition of tolerance.

Dr. Dews: The mammalian brain may not be the best place to start.

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*Ed. Note: Recently, Seevers reported (1968) that he and Shideman were unable to repeat this finding in subsequent experiments.

Perhaps we should start with something like a sea snail (Tauc and Kandel, 1964).

Dr. Wallgren: On the other hand physical dependence cannot be demonstrated in very simple organisms.

Dr. Kalant: Has physical dependence not been demonstrated in paramecia, with respect to ethanol?

Dr. Wallgren: Tolerance has been shown; but not physical dependence.

Dr. Kalant: In the experimental morphine withdrawal which you mentioned, Dr. Domino, did the muscle show an *increase* in oxygen consumption?

Dr. Domino: Yes, it was an increase in oxygen consumption.

Dr. Kalant: Was this accompanied by a study of the electromyogram?

Dr. Domino: No. This was an *in vitro* study.

Dr. Wallgren: But it may be related to events in the nervous system. The characteristic features of withdrawal of alcohol, barbiturates and opiates are various neurological manifestations of hyperexcitability, and this must involve the central nervous system, as Seevers has shown.

Dr. Domino: Even in the case of the opiates further control data are necessary, since we do know that organisms adapt to a variety of chemical substances. The crucial point is evidence of withdrawal symptoms. Even in the case of the morphine studies in tissue cultures, it is essential to use other narcotic-like agents that are nonaddicting, such as thebaine, for comparison with morphine. This has yet to be done. I am not sure we can really say physical dependence has been demonstrated even in tissue culture.

Dr. Quastel: I do not think this is an impossible thing. You can get dependence by bacteria. I suppose it is probably a general phenomenon.

Dr. Kalant: What about the work with opiates in cultures of E. coli (Simon, 1964)?

Dr. Titus: I think he was trying to demonstrate the appearance of dependence in E. coli, but unfortunately morphine did not affect the cells of this organism. The more toxic levorphan did inhibit growth, apparently by inhibiting the synthesis of ribosomal RNA. It did not inhibit DNA synthesis, and with special mutants it could be shown that inhibition of protein synthesis was secondary to inhibition of RNA synthesis. I do not think he actually demonstrated dependence in this organism.

Dr. Brazier: What are the signs of dependence in these lowly organisms

Dr. Titus: With microorganisms it would be the dependence of growth rate on the presence of the drug. In the tissue cultures, the morphology changes, unless you present the drug.

Dr. McIlwain: It is certainly true that one can adapt a microorganism to require a compound normally toxic. This was shown with sulfonamides; sulfanilamide normally inhibits growth, but in certain instances, by a resistant growth one can develop a strain of organisms which actually requires sulfanilamide.

Possible Sites of Changes Involved in CNS Adaptation

Dr. Titus: But this can be genetic selection too, and this is a different matter. I would be interested to know what molecular changes you think might be involved in these adaptations in the central nervous system. Assuming for the moment that they do not involve inductive effects and synthesis of new protein, there must still be some molecular alterations which occur as a result of passage of an electrical impulse. Would you care to speculate what such changes might be?

Mitochondrial Induction

Dr. McIlwain: There is some indication, for instance, that mitochondria are found in greatest numbers in an area where there is greatest call for energy-rich materials; at the nodes, for instance, in peripheral nerve. If there has been an extreme excitation of some pathway which has involved a great utilization of this synaptic pathway, why should there not be a greater number of mitochondria induced? How you induce a greater number of mitochondria, I just do not know; but one idea is that subdivision can occur. When you have a given number, what maintains that number? Again, I do not know. But once there have been five mitochondria at a particular spot, there may be some mechanism for maintaining five mitochondria there. As you see, this can only be quite speculative.

Also, a key point must be the actual synapse, a point of apposition between the parts of two cells. This is a highly structured region and physically a very tough one. If you tear up the tissue, you are still left with the point at which these two cells were brought together; there is some particularly well engineered and physically resistant material here. But once this has been disturbed, in the sense perhaps of a greater rate of cell discharge than normal, perhaps some change occurs to maintain this new rate. Now what does firing consist of? It consists possibly of the release of acetylcholine from vesicles. What is the picture of this release? It involves a fusion of the membrane which has been around the vesicles to the membrane which is now at the synapse. In other words, there may be a material alteration at this highly engineered piece of structure, which may subsequently permit easier fusion of the vesicles to the synaptic membrane. It is this sort of idea that one might picture, and I think such a change might be independent of protein synthesis. Now whether there are means of maintaining these changes other than the sequence of RNA to protein synthesis which is well worked out, I just do not know. And I prefer to maintain a quite open mind on this.

Synaptic Alterations

Dr. Kalant: In that connection it may be appropriate to mention the work of McQuarrie and Fingl (1958). They reported that the administration of a single dose of ethanol to mice elevated the threshold of electrical stimulus necessary for production of a motor convulsion; but following the disappearance of the ethanol, several hours later, there was a period of hyperexcitability, i.e., abnormal lowering of the threshold, followed by a later return to normal. The hyperexcitability was more intense and more prolonged, the longer the period of previous ethanol administration and the higher the dosage of ethanol has been. This work does not suggest any difference between the type of withdrawal reaction following the single dose of alcohol and that following prolonged administration of ethanol. I think this would tend to favour a functional change which was independent of any major change in protein synthesis.

Functional Changes

Dr. Quastel: Perhaps I might venture a hypothesis and comment? I cannot believe that every one of the thousands of millions of neurons in the brain is functioning at an optimal rate; it seems probable that many of the billions of neuronal pathways may be regarded as dormant, not functioning. Now if we accept this as a working hypothesis, we may imagine that administration of a drug, such as ethanol, would

Concept of Dormant Neuronal Pathways

result in the block of several of the normally functioning neuronal pathways. Then, if the usual biological mechanisms apply, a compensatory change may take place, as occurs in the vascular system after interference with the circulation. We may expect, during the chronic administration of ethanol, the opening up of new neuronal pathways; that is to say, pathways previously dormant may now become functional. If this does occur, then presumably a further dose of alcohol may be required to block the newly functioning pathways, and this could go on, more and more alcohol being required to suppress the newly developed neuronal pathways.

All this speculation, of course, depends on the idea that there are dormant neuronal pathways in our brain that are capable of becoming functional when the existing ones cease to operate optimally. It would follow that if the drug is then removed, all these various neuronal pathways that have been opened up, together with the older suppressed ones, now become functional. There should result a very considerable hyperactivity. This is, I realize, a rather simple way of looking at thing but I would like to know of some experimental method by which we could discover whether there is any truth in the hypothesis or not. It seems to offer one way of understanding the processes of addiction and of withdrawal . . . . . two phenomena which, I believe, are intimately connected.

Addiction vs. Dependence

Dr. Kalant: This is a point which has received a good deal of attention recently. The World Health Organization Expert Committee (1964) recommended the dropping of the term 'addiction' and the adoption of the term 'dependence', because of the argument about whether or not withdrawal phenomena are an intrinsic part of the process of addiction. In this context, Nichols (1965) differentiated between addiction and dependence produced experimentally in rats. Dependence and withdrawal symptoms could be induced by parenteral administration of morphine. But addiction, i.e., the voluntary ingestion of large and increasing amounts, could be induced only by suitable manipulation of the schedule of administration such that the rat learned to ingest the morphine solution voluntarily, to suppress withdrawal symptoms which followed the parenteral injections. In other words, addiction required an active behavioural component as well as tissue dependence.

Dr. Dews: Analogies to phenomena of supersensitivity come to mind. If you keep an animal under the influence of atropine for a while, so that the effects of the nervous impulse are not transmitted to the

Supersensitivity Phenomena

secretory cells of the salivary gland, after a while the salivary gland becomes more sensitive to stimulation of secretion by adrenaline. The mere deprivation of a cell as crude as a salivary gland cell, for a period, from its normal supply of excitatory impulses induces this cell to change so that it becomes more sensitive to stimulation through other available mechanisms. You cannot use acetylcholine to stimulate the cells in the presence of much atropine, but the denervated salivary gland, of course, becomes supersensitive to acetylcholine. If a cell of the central nervous system is deprived of its normal quota of excitation by virtue of the effect of a drug such as morphine, why should not this cell become supersensitive? When morphine is removed and the cell again receives its original quota of bombardment, we would expect it to discharge excessively. Remember that the phenomena of withdrawal generally affect the same systems that are initially affected by morphine, but the effect is opposite in direction.

Dr. Domino: But if you give chronic treatment with atropine you do not get atropine withdrawal symptoms, do you?

Dr. Dews: I do not know. But it takes several days for the effects of atropine to disappear, and the decline of supersensitivity may be able to keep pace. The situation may be likened to the lack of serious withdrawal symptoms following phenobarbital, which we discussed earlier (pp. 108-110).

Dr. Kalant: It appears that we have very little factual information concerning the cellular processes that underlie the acquisition of tolerance, dependence, and withdrawal reactions. Would anyone care to discuss the relation between addiction and the phenomena of tolerance and dependence?

Change between Acquisition and Maintenance of Addiction

Dr. Hoff: Dr. Russell, in talking about maintenance or persistence of addiction, proposed one mode of looking at this question. But I was concerned that he did not think it might ever be possible to define or to determine cellular or biochemical changes that would modify the secondary stimulus drives; if this could be done, it would be very helpful. Why does the individual continue with his addiction in spite of the fact that there are certain punishments, certain damaging effects to the individual, that result from it? The addict continues even after the primary drives seem no longer to be satisfied. For instance the relief of pain; taking the drug may now cause him more pain, or its psychological equivalent, than it relieves. So the picture changes between the acquisition of the addictive behaviour, and the maintenance of it. I can see how the addiction might be acquired; but why is it maintained when the maintenance is apparently so costly to the integrity of the organism?

Dr. Russell: There are two major directions in which to seek answers to this question. First, factors leading to the formation of the behaviour in the first instance may be functioning at even a higher level than they did during the acquisition. For example, anxiety may initially lead to drug-taking as a means to obtain relief. But this practice, being contrary to socially accepted behaviour, may raise the anxiety to even a higher level than existed during the acquisition of the practice. The individual continues to make the drug-taking response as a means of anxiety reduction. A second possibility is that the process that we refer to as dependence involves some tissue change which in itself serves as the basis for the continued maintenance of the behaviour: a new 'basic drive' has been induced. In order to control effects which are produced by the return of the tissue conditions to their normal pre-drug state, the drug-taking behaviour continues.

Dr. Hoff: Yes, the addict continues to take it because he now uses it as a medicine to relieve some of these damaging effects. But he is also presumably taking it to gain the same satisfactions as before, although he may find that these are less and less readily produced. The question is, why does he not stop, if he gets to that point?

Dr. Kalant: In effect, you postulate that he does not stop, because stopping would precipitate new painful stimuli dependent on withdrawal. To this extent, addiction and tissue dependence are clearly related. But how does one explain that if he is stopped by hospitalization or other treatment, and any measurable tissue changes, as manifested by withdrawal symptoms, disappear, he so often resumes the drug-taking later?

Conditioning as a Factor in Addiction

Dr. Russell: I suggested earlier that this might be accounted for in terms of conditioning to extraceptive or intraceptive stimuli which occurred during the acquisition and maintenance periods. Stimuli which had been associated with withdrawal and, therefore, led to maintenance of the habit, could, if they arise again *from other causes,* set off the drug-taking response. It is also possible that the re-establishment of the habit, after withdrawal is completed, may be explained in terms of the same conditions which were involved in its original acquisition.

Dr. Dews: I would like to put a question the other way round, although it is still the same question and has not yet been answered. It seems to me that if you say that morphine is a powerful reinforcer, because behaviour leading up to the administration of morphine is strongly maintained, then the question becomes: what is it about morphine that makes it a powerful reinforcer? How can it take over from other reinforcers? Now the problem is of the same nature as 'how can electrical stimulation of the hypothalamus function as a reinforcer?' We are back in a biological context, rather than thinking about addiction in terms of 'why does he do it, when he should know better?' I would like to look at it in a biological way rather than in terms of the supposed reasonings within the addict, because I do not think there are rational processes involved. It is an automatic, mechanistic sort of thing.

Reinforcement in Addiction

I think that in talking about why addiction starts, in the first place you have got to ask: what is the concatenation of circumstances that makes even an initial dose of morphine reinforcing? To most people under normal circumstances it is not. Studies and reports of many people's personal experience indicate that the initial dose of morphine is anything but reinforcing. Most people feel quite wretched and will not readily take it again (Beecher, 1955). One of the ways in which even an initial dose of morphine can be made reinforcing is to have an individual in severe pain; then morphine has a powerful reinforcing effect. Another way is presumably to put an individual in bad social circumstances, where he is miserable and has no other powerful reinforcer available, nothing else to keep him going; he has no job; he has not any interest; morphine has nothing to compete with, so to speak. He comes in contact with a group which is using the drug and from the very beginning the drug starts to take over as a reinforcer. It does not have to compete with any other powerful reinforcers, so that even if it is weak to begin with, it has not much competition and so the insidious process gets started.

Once it does start, then one of the characteristic features of progressive addiction is that addicts tend to move from the slower means of onset of drug effect to the more rapid, and eventually to intravenous administration. One of the things we know about the process of reinforcement is that the more rapidly it follows behaviour, the more powerfully it reinforces that behaviour. When someone takes morphine intravenously, therefore, there is very powerful reinforcement of antecedent behaviour which is much more difficult to interfere with than in the initial stages. Looking at it from this point of view, I have more hope of being able to do experiments.

Dr. Kalant: This is relatively easy to follow, I think, with respect to a single drug, and the progression from subcutaneous to intravenous administration, or the increase in dosage as tolerance is acquired. But on this basis, how does one explain the frequent observation that a patient may transfer back and forth from one drug to another, more or less indifferently? (see also p. 190). There are recorded cases of patients switching from ethanol to amphetamine; the pharmacological effects of the two drugs are presumably quite distinct, yet there is apparently a similar type of satisfaction of whatever drive leads the person to use the drug in the first place. Is the reinforcement non-specific?

Drug Substitution by Addicts

Dr. Brazier: Can one avoid withdrawal symptoms if the drug that caused the addiction is withdrawn and some substitute given?

Treatment of Withdrawal

Dr. Dews: Yes. Administration of methadone will prevent morphine withdrawal symptoms. As a matter of treatment, the methadone must then be withdrawn, but the methadone withdrawal syndrome is much less florid than the morphine withdrawal syndrome.

Dr. Brazier: Our biochemical discussion has related only to a search for a tissue change that is general. Dr. Russell, I think, talked about the maintenance of drug-taking being due in part to tissue demand. Well, if there is a tissue demand, is it a specific one? Does that mean a demand for *this specific drug,* or is it a generalized non-specific tissue demand? If so, you should be able to stop withdrawal symptoms by giving any of a large number of drugs.

Dr. Kalant: Surely the best evidence concerning this is that alcohol withdrawal symptoms can be alleviated, with varying degrees of effectiveness, by tranquillizers, or barbiturates, or chloral, or paraldehyde, or various other drugs. They are to that extent interchangeable.

Dr. Domino: For controlling ethyl alcohol withdrawal symptoms, paraldehyde is the best, while drugs such as chlorpromazine and promazine, though useful, do not do nearly as effective a job. In other words, the closer the substitute drug is to alcohol, in this case paraldehyde, the better it suppresses alcohol withdrawal symptoms.

Dr. Himwich: That carries a danger with it because then paraldehyde produces dependence too.

Dr. Domino: The reason it is being returned to clinical use is that the incidence of alcohol withdrawal convulsions is much smaller with paraldehyde than with promazine or chlorpromazine therapy.

Dr. Hoff: In the section of your paper dealing with acquisition of drug-taking behaviour, Dr. Russell, you mentioned anxiety as a secondary drive, and you mentioned the animal in the shuttlebox who was shocked in the one section and ran to the other section.... (see p. 27).

Dr. Russell: I was trying to define 'anxiety' in objective behavioural terms: in terms of a stimulus which has acquired the property of initiating behaviour through its association with some noxious stimulus itself.

Aversive Techniques

Dr. Hoff: If one assumes one prevalent idea about the acquisition of addiction or dependence to alcohol, anxiety arises as a live issue. One of the facts, speaking clinically, is that anxiety is so rapidly relieved by alcohol that there is a very strong and close relationship between the anxiety and the taking of the alcohol that abates it so quickly. The punishing effects, the withdrawal symptoms, come later and are not locked in time so closely to the secondary drive stimulus. I wonder whether this observation provides a lead for behavioural research? Some clinicians have tried to make a therapeutic objective of tying in closely the punitive results, the damaging effects, with the taking of the alcohol. This is the basis of the aversive techniques. Can one study the addictive process experimentally in those terms?

Treatment in Acquisition and in Maintenance Phases

Dr. Russell: It is important to distinguish between the acquisition phase, in which anxiety could be one of the processes of reinforcement involved, and the maintenance phase. I suggested earlier that two different processes are involved: that, as a result of drug-taking during the acquisition phase, there develop certain persisting tissue changes that then serve as the basis for the maintenance of the habit. To affect the drug-taking behaviour in each of these phases would require quite different methods. During the acquisition phase we would seek ways, other than drug-taking, of relieving anxiety.

During the maintenance phase, we would employ a combination of chemotherapy to handle the tissue changes induced during the acquisition phase and, at the same time, introduce some behavioural therapy to eliminate any conditioning of extraceptive or intraceptive cues that might result in reestablishment of the drug-taking behaviour. If different processes are involved, as would seem to be suggested by behaviour during the two phases, then we would approach the therapy as if there were two different problems.

Dr. Hoff: Prevention of addiction, in other words, is a different problem from therapy.

Drug Transfer

Dr. Kalant: I wonder if this is also the answer to the question I raised before (p. 188) about the explanation of a transfer of drug use from barbiturates or ethanol to amphetamines. The tissue dependence phenomenon might be involved only in the maintenance of intake during the period in which tissue tolerance has developed. If the process is interrupted at some point by a temporary period of abstinence, so that the tolerance and dependence upon a particular drug have disappeared, gratification of the conditioned drives might be achieved then by a totally different drug. One might settle the question by looking at individual case histories, to see whether or not there is a gap between the taking of the two drugs, or whether one drug can directly supersede the other.

Dr. Brazier: Is there not a big change in overt behaviour of the person? I am not talking about the rat in the maze, but the human users. There must be a big change in their overt behaviour when they switch from a barbiturate to an amphetamine, surely?

Dr. Kalant: Oh yes, a very big change. Zondek (1958) described the case of a marine engineer who was a very good worker on the ship but inevitably became drunk as soon as he landed. He consulted a doctor who prescribed an amphetamine-barbiturate mixture, and from that time on he never drank; but he did continue the amphetamine to the point of developing a chronic amphetamine intoxication. There was certainly a change in behaviour, but the compulsive intake of a drug was the same, except for a switch in the drug employed.

Recently I read, in a non-scientific but apparently reliable source, that in some parts of South America alcoholics have made use of cocaine for getting over severe hangovers and withdrawal symptoms, and have then transferred their dependence from alcohol to cocaine.

Dr. Dews: Is it not true that if morphine addicts cannot get hold of morphine for any reason, they will take barbiturates rather than nothing?

Dr. Himwich: Or an antihistamine.

Dr. Dews: They will take anything they can get. There is a non-specific component.

Dr. Quastel: A placebo would not do?

Dr. Himwich: I do not think so. It must be a drug that does something.

Dr. Russell: You would not expect a placebo to help during the maintenance phase.

Dr. Himwich: No. The placebo will do everything that a placebo *can* do, and it *can* do certain things, but it cannot supply all that is necessary to the addict.

F. STUDIES ON POSSIBLE PREDISPOSING FACTORS IN ADDICTION DEVELOPMENT

Dr. Hoff: In talking about drives, you spoke about reduction of a need, or some kind of satisfaction or reward to maintain the drive. I was wondering about imprinting. Is it possible that this may be another fruitful area to explore in terms of acquisition of addictive behaviour?

Imprinting and Drugs

Dr. Russell: Imprinting is an interesting phenomenon that involves very rapid learning at critical periods during early development. Much research on the nature of the phenomenon has been done by comparative ethologists, such as Baerends, Hartley, Lorenz and Tinbergen (see Hinde, 1966). Hess (1960) studied the effects of meprobamate, chlorpromazine and pentobarbital on imprinting, comparing them with placebo controls. The particular form of behaviour studied can be established in the chick or in the mallard duckling at a certain critical age, 13-16 hours after hatching. The behavioural pattern was induced by presenting previously isolated ducklings with a decoy model of a male mallard duck; as the model moved, the ducklings followed it. When tested at later ages, the subjects would follow the model selectively. The three drugs tested had quite different effects upon the imprinting process. But the important point is that a form of behaviour induced during a *limited critical period* in early development persists for long periods thereafter.

Dr. Hoff: Yes, and it does not depend on repetition.

Dr. Russell: All that is needed is a short period of exposure to a previously neutral stimulus and the behaviour pattern then persists. But the association must be established during the critical period. After this age, 'imprintability' declines and, at about 20 hours, avoidance, rather than following, becomes the response to any moving object.

In the drug experiment I was describing (Hess, 1960), the hypothesis was that reduction of emotional responses, 'fear' and its accompanying 'anxiety', by drugs would prolong the critical period. Effects of three drugs were studied: meprobamate, 25 mg/kg; pentobarbital, 5 mg/kg; and chlorpromazine, 15 mg/kg. When the drugs were administered just prior to the critical period, at the twelfth hour after hatching, the results showed that meprobamate reduced the emotional behaviour, but made imprinting almost impossible. Chlorpromazine was associated with a high degree of imprinting and extended the critical period. Pentobarbital did not affect the emotional responses, but did reduce imprintability. The results of this experiment illustrate the *differential effects* of drugs upon behaviour *during critical periods* in early development. There is accumulating evidence for the fact that alterations in the biochemical environment *in utero,* and during the first few days after birth, may produce lasting changes that will affect capabilities to learn and emotional behaviour for months and even years thereafter.

Dr. Brazier: Has imprinting, as differentiated from training, been shown in any phyla higher than the birds?

Dr. Russell: Most experimental work on imprinting has, so far, been carried out with birds. However, the phenomenon has been demonstrated with guinea pigs and sheep. An attempt has been made to study imprinting in the human infant, using the 'smiling response' as the motor equivalent of the 'following response' in infrahuman animals (Gray, 1958).

Dr. Brazier: Does not Harlow call his 'terry cloth mother' phenomenon in baby monkeys a training phenomenon, rather than imprinting (see Brazier, 1960)?

Dr. Russell: Yes. Imprinting in monkeys has not been studied directly. However, behavioural deficiencies in adulthood have been found in monkeys reared with artificial mothers.

Dr. Brazier: Hess and Lorenz, and Tinbergen and Thorpe all work on birds, phyla that have no neocortex (for references, see Brazier, 1960). Has this anything to do with it?

Dr. Wallgren: I think it might well have. Generally, birds' behaviour is characterized by much stereotypy which is not adaptable, but mammalian behaviour is much more plastic and adaptable; and it has been suggested that this is related to the relative development of the neocortex.

Dr. Himwich: There is a division in neurophylogenesis after the reptiles, the birds going more to subcortical development, but with some neocortical, too; and in the other direction, mammals preferentially developing the neocortex.

Dr. Kalant: If one differentiates imprinting from later learning or training, and if imprinting occurs only in certain phyla and only at certain very critical stages early in life, are drug effects upon it likely to yield relevant information with respect to the acquisition of a behaviour pattern like addictive intake of drugs?

Dr. Russell: Perhaps I can answer that question by saying that I personally would not center a research program on the phenomenon of imprinting if I were interested in the larger problem; such research would, of course, be interesting in and of itself. There is still argument as to whether or not imprinting involves the same basic process as learning. Ethologists take the view that it is much more concerned with genetically determined processes, whereas others have argued that this conclusion is not warranted unless it can be absolutely established that there is no opportunity for learning to account for the appearance of the behaviour. But to return to your basic question, I would say that imprinting is not a preferred kind of behaviour to study as a model for the acquisition of addictive behaviour. I think it would be much more interesting to study effects of drug administration early in the individual's development.

Developmental Vulnerability

Dr. Hoff: Do you remember the work of Witkin *et al.*, (1959) (see also Karp *et al.*, 1965), on field dependency, in which he studied alcoholics and non-alcoholics in a tilt room? The subject was put into the room, seated in a chair which he could control. He was asked to keep his eyes open while the room was tilted, and to operate the chair controls until he felt that he was seated in the vertical position. Witkin found that alcoholics made consistently greater errors in this than did other psychiatric patients or students. He built a hypothesis that alcoholics were more visually field-dependent than other persons. This suggests that in the development of the individual some persons remain more passive than others, more visually dependent and less trusting of their proprioceptive system. This may be the result of some trauma that occurred at about the time that the child was sitting up and beginning to stand and walk. I wonder if this is a behavioural approach to the problem of acquisition of addiction. This relates to the possibility of developmental vulnerability as an etiological factor in the development of the addiction.*

Personality Factors

Dr. Russell: This is a whole area of research which is also very important: what preceding factors in the individual's life history may have predisposed him to be more susceptible to developing addiction habits? There has been some research done at the level of personality factors, as assessed by such instruments as the Minnesota Multiphasic Personality Inventory. In general the finding is that the addict may show deviations from normal, but these deviations are also found in samples of nonaddicted subjects.

Dr. Dews: Have any of these studies been done in a prospective manner?

Genetic Factors

Dr. Russell: None that I know of. It is the way in which they ought to be done, but it is an expensive and time-consuming approach. There are other ways in which information about predisposing factors may be obtained. In searching for possible genetic factors, we might ask, for example, what are the effects of strain differences? Comparisons of different strains of rats or mice could be used in studying their relative susceptibility to the development of experimental addiction. I do not know whether any of the methods of analysis in human genetics have been applied to problems in this general area.

Dr. Hoff: I think not. Rodgers *et al.* (1963) have studied different strains of mice, and find that a higher tolerance to alcohol goes together with a higher alcohol dehydrogenase activity in the liver and kidney.

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*Ed. Note: The validity of Witkin's interpretation of studies of field dependence in alcoholics has recently been challenged by Kristofferson (1968). The findings may result from impairment of proprioception, as a secondary consequence of alcoholism.

Dr. Russell: More research of this kind should be done. It might also give us some good leads as to where to look for the nature of tissue changes associated with the phenomenon of dependency.

Dr. Kalant: The inter-strain differences in intake which Rodgers *et al.* (1963) found were not of a magnitude such that one could consider one strain alcoholic and the other nonalcoholic. The differences in intake, though highly significant, were determined by the amounts which the animals could metabolize, so that there was no evidence of any appreciable blood level or of any central nervous system effect. Dr. Wallgren has been closely associated with Dr. Forsander and others, in comparative studies of alcohol intake by different species and strains; perhaps he may have some comment about this.

Alcohol Preference in Rats

Dr. Wallgren: Mardones *et al.*, (1953) have done similar work on rats; and their work, also, did not turn up any animals with a very high intake of alcohol. They could differentiate and actually select two strains, one of which had a very *low* intake of alcohol when presented with a free choice between alcohol solution and water.

We started out in 1956 with rats of an original Wistar strain, and at that time they did not voluntarily drink very much alcohol. The usual situation has been to give them a free choice between 10% alcohol solution by volume, and water. In the beginning they took about 25-30% of their total drinking fluid as alcohol solution, and the remainder was water. But over the years we have been seeing increasing numbers of rats with a very high spontaneous intake of alcohol in this situation. Now we have a very wide spectrum; there are actually very few rats taking little alcohol; there are some individuals taking only about 10% of the total intake, but quite a number are drinking somewhere around 50-50; and a surprisingly high number are drinking 80-90% of the total intake as alcohol solution.

Dr. Hoff: For how many generations have you had this going?

Dr. Wallgren: Over nine years now; that must be about 18-20 generations. Now we have started a genetic selection experiment, because we have this rather unique material. We are attempting to select two different strains, one with a high intake and the other with a low intake.

Dr. Russell: You are selectively interbreeding animals with a high and with a low intake and trying to . . . . .?

Dr. Wallgren: Yes, trying to separate them. But there is one quite interesting point in these animal series. We do not claim that the high-intake animals are alcoholics, but they do consume spontaneously about 10-11 grams of ethanol per kilogram of body weight over a 24-hour period; and that, of course, mainly during the night because rats are active during the night. We thought there must definitely be an appreciable amount of alcohol in their blood sometime during the 24-hour period; and by taking blood from them about 4 or 5 a.m., we actually found well above 0.1% of alcohol in the blood. That is, of course, not very much for a rat, because rats tolerate alcohol so well, but it is sufficient to give some overt behavioural effects, at least in a rat which has not acquired further tolerance.

Dr. Hoff: Have you done any enzyme studies on those animals?

Metabolic Studies in High Preference Strains

Dr. Wallgren: The studies that have been done so far were not concerned with alcohol dehydrogenase; but we are starting work on the elimination rate. Another interesting point here is the work which has been done by Forsander and Salaspuro (1962; see also Forsander, 1966) on the metabolic alterations induced by a challenging dose of ethanol. We know that ethanol alters the pattern of liver metabolism very much. Ethanol blocks almost completely the use of all other substrates. The oxidation of ethanol gives the liver almost all the energy it requires as long as alcohol is present, and it apparently blocks the citric acid cycle to a very large extent. The intrahepatic oxidation of ethanol goes only to the stage of acetic acid, which then passes out in the blood.

Dr. Himwich: Is further oxidation of acetic acid to CO_2 and H_2O blocked?

Dr. Wallgren: Yes, hardly any carbon dioxide is formed in the liver, even though the oxygen consumption stays normal. There is a great change in certain metabolites, reflecting a more reduced condition in the liver; we get a higher level of lactate and often a very large change in the lactate-pyruvate ratio.

Dr. Quastel: A high level of NADH?

Dr. Wallgren: Yes, also that. We think the ratio of lactate-pyruvate is more important, since the German workers (Bücher and Klingenberger, 1958; Hohorst, Kreutz and Bücher, 1959) have shown that the NAD/NADH ratio is not a very good indicator of the redox state because so much of the nucleotide is bound. The lactate-pyruvate ratio is a better reflection of the intracellular (cytoplasmic) redox state, and usually we get a big difference with alcohol. There is not a very great increase in lactate, but pyruvate tends to go down a little also, so that usually there is a large change in the ratio. But when they started to compare the high and low intake animals, Forsander and Salaspuro found that in animals having a very high spontaneous alcohol intake, a challenging dose of alcohol produced only a very small change in the lactate-pyruvate ratio; whereas in the rats drinking little alcohol, a test dose could cause anything from a very small to a very large change in ratio. In some, the changes were tremendous: the ratio increased by a factor of something like 10 or 20, compared with the control ratio.

Dr. Quastel: Perhaps the alcohol is not the main nutrient in the animals with little change in ratio.

Dr. Wallgren: No. We do not know what the reason is, because alcohol actually can be oxidized at approximately the same rate, but still it has a very different effect. We do not know why this is . . . .

Dr. Quastel: Is it not a fact that with these animals that are drinking large quantities, the actual rate of oxidation of alcohol by the liver is pretty much the same?

Dr. Wallgren: Yes.

Dr. Himwich: Does alcohol dehydrogenase activity rise in the liver if alcohol is taken chronically?

Dr. Wallgren: Yes, but not very much. . . .

Dr. Himwich: I believe that Greenberger *et al.* (1965) found little or no increase.

Dr. Kalant: We find an increase of about 50-60% on daily administration for 2 months in rats (Hawkins *et al.,* 1966; Khanna *et al.,* 1967).

Dr. Wallgren: Yes, that can happen. We do not really know yet whether chronic administration will raise the rate of elimination, but there is evidence that it is very possible.

Dr. Kalant: We have found that the increase in slope of the blood alcohol disappearance curves in chronically treated animals parallels the increase in liver alcohol dehydrogenase activity.

Dr. Wallgren: Yes; but I think a very striking point here is that the other alterations in metabolism induced by alcohol are much smaller in the animals which voluntarily drink a lot. Forsander interprets this as a factor *permitting* the animal to drink alcohol but not necessarily *inducing* it to do so; and the animal in which we have a very great metabolic disturbance induced by the challenge dose of alcohol, we picture as not being capable of drinking any appreciable amounts of alcohol, which they do not do. But there is a great variation in the metabolic effect of alcohol in the animals with a low intake, which suggests that other factors besides the metabolic ones influence the final behaviour towards alcohol.

Dr. Quastel: Do the animals change weight? Are they getting any nitrogen?

Dr. Wallgren: Oh, yes, they are on a normal diet, and there is actually no difference in weight.

Dr. Himwich: This is an immediate result, or after several generations?

Dr. Wallgren: These are the results we have obtained with the rats as they are now. I think the interesting point here is that there is an apparent relation between metabolic factors and the preference for alcohol.

Dr. Russell: That suggests a related kind of experiment which I would like to see going along with your studies. It would involve applying Weeks' or Nichols' procedures for producing dependence in rats in order to measure individual differences in the amount of alcohol and in the period of time necessary to produce dependence. Then, by selectively breeding animals from each end of the distribution curve of individual differences, it would be possible to determine whether or not there was a genetic factor involved in susceptibility to the development of dependency. The same experimental approach might be used with respect to withdrawal and other behavioural characteristics of addiction.

Dr. Wallgren: In relation to alcohol, I think if we succeed in separating these strains, we will have enough material to let us work from many angles, including, of course, a study of the development of tolerance and disappearance of tolerance. Also, while I do not think anyone has induced real symptoms of physical dependence on ethanol in rats, we have the partial finding by McQuarrie and Fingl (1958) on increased nervous excitability on withdrawal in the mouse.

Dr. Kalant: But that was by forced administration, not by voluntary oral intake. Dr. Wallgren, do the animals which have a high voluntary intake of ethanol, and which do not show much change in lactate-pyruvate ratio after a test dose, show a depression in carbon dioxide output such as you observed with the normal animals, or do they continue to show an essentially normal respiratory quotient?

Dr. Wallgren: I do not think that was measured in these same experiments. Liver perfusion experiments have shown rather a uniform effect in suppression of carbon dioxide output. This has not been specifically investigated in *these* animals, because Forsander and Salaspuro did not do liver perfusion in this work; they gave an alcohol dose and then removed the liver, processing it as fast as possible, to get the average levels in the different groups.

Dr. Kalant: One wonders if the process of selection of high-intake rats over the years has involved the appearance of a larger quotient of mitochondria per liver cell? Is their citric acid cycle greatly increased in capacity? Do they perhaps show a higher stimulation by succinate, for example, of respiration by slices of their livers?

Dr. Wallgren: Yes, possibly. I think it is important to remember that the oxygen consumption is rather similar in the different groups. It is the carbon dioxide production that is decreased. We do not really understand why they are capable of oxidizing such large amounts of alcohol. Certainly spontaneously they do not drink more than what you can account for on the basis of an average rate of alcohol elimination. But even this rate is so high, that it could supply a very large proportion of the energy required. Then there is evidence that in certain instances the metabolism of ethanol goes up much higher. Well, why does it happen? – Because even at the lower levels it should supply all the energy that the liver requires. Is there a peripheral system induced which can utilize ethanol? We do not know.

Dr. McIlwain: What is the ultimate fate of the carbon? Is it oxidized by other organs of the body?

Dr. Wallgren: Yes, the acetate and active acetate are metabolized mainly by other tissues, and ultimately more than 90% of the alcohol comes out at CO_2.

Dr. McIlwain: This is a change in metabolism in the direction of that in ruminants, which have an appreciable blood level of acetate.

Dr. Domino: When Dr. Wallgren talked about lactate-pyruvate ratios it reminded me of investigations concerning lactate-pyruvate ratios in certain schizophrenics, whose plasma causes a rather high ratio in chicken red cells incubated in it. I would like to ask whether there were any obvious behavioural differences between the rats that had high alcohol ingestion and relatively low lactate-pyruvate ratios, and the other group of rats that did not take much alcohol and had very high lactate-pyruvate ratios?

Dr. Wallgren: We do not really know. That is one of the things we want these two strains for. We are actually experimenting with behavioural tests by which we hope to be able to characterize the rats and correlate their differences with differences in drinking and metabolic characteristics; but so far we have no behavioural data on these rats.

Alcoholism and Schizophrenia

Dr. Domino: My other question is directed to those who are clinically oriented. Is there any relationship between chronic alcoholism and schizophrenia?

Dr. Hoff: Yes. We are now making a study of our first 7,000 patients and many of these patients are schizophrenic. I would say this: if you take all of the patients and the psychiatric diagnoses that could be assigned to them, you have quite a broad spread of diagnostic items.

Dr. Domino: What I was trying to get at is a metabolic relationship. This is probably far-fetched, but the sort of prediction I would like to make would be that the incidence of schizophrenia among alcoholics is less than the incidence of schizophrenia among nonalcoholics. This possibility arose in my mind as a result of some of the findings that Dr. Wallgren mentioned about lactate-pyruvate ratios in his rats.

Dr. Hoff: When we have finished analyzing our patient data, we hope to be able to tell you that. Another suggestion that has been made is that the alcoholic may have a genetically determined excessive rage response, which he normally keeps under strict obsessive control, and for which drinking may provide some sort of release.*

Dr. Russell: I should think that genetic factors certainly might be involved, and this is why I would like to see some selective breeding studies of the kind we were discussing.

It would also seem important to investigate the possible involvement of early environmental factors. For example, suppose that in addition to selective breeding studies of susceptibility to acquiring addictive behaviour, or of its extinction, we also studied the effects of exposure to different amounts of experimental frustration or conflict preceding the acquisition process. Would we find that susceptibility is related to the amount of prior frustration or conflict? There are a number of experimental approaches by which we could get information about the influences of genetic and environmental factors on the acquisition, maintenance and extinction of addictive behaviour.

Dr. Brazier: Does Dr. Hoff think it unusual for a schizophrenic to become an addict? In the days before tranquillizers, when you walked down the floor of a mental hospital, you could smell the paraldehyde and the chloral hydrate, but you were always told that these people did not become addicts, although they were being given these drugs for long periods of time quite intensively. Is this so, Dr. Hoff?

Dr. Hoff: To be truthful, I have never given any thought to the question of whether a schizophrenic would be more or less likely to become alcoholic.

Dr. Himwich: In the realm of speculation, I would suggest that there would be relatively more alcoholics among schizophrenics than among non-schizophrenics, because borderline schizophrenics may take alcohol to help them adjust to the environment.

Dr. Kalant: On this note of speculation, we shall unfortunately have to end our present discussion. I would like to say again, on behalf of the Foundation, that we are very grateful to all of you for coming here and exchanging freely your ideas and suggestions. There are many other points which might have come up here, if time had permitted. It is our hope that many of them *will* come up in future conversations and meetings, and that the full value of this trade of thoughts across the boundaries of several different disciplines will be found in its effectiveness as a stimulus to future experiments.

*Ed. Note: See Fleetwood (1955).

Epilogue

During the course of the conference it became clear that no comprehensive statement of contemporary expert opinion on the nature of the addictive process could be reached to which all participants could subscribe. There were too many obvious gaps in knowledge to permit such a statement. Further, the gulfs between areas of behavioural, physiological and biochemical knowledge were too wide to be bridged by mechanistic explanations. Nevertheless, a surprising measure of agreement was reached with respect to methodological difficulties and requirements common to all three disciplines, points which could be accepted as reasonably well proven fact, and areas which required intensive research. Some of these are summarized below.

Questions of Methods and Terminology

(a) Choice of test measures. At all levels of investigation of drug actions, there is a widespread failure to define adequately the basic components of biological processes upon which drugs may be expected to produce changes. This failure is perhaps most evident in behavioural studies, many of which have employed only a single criterion of performance (e.g., maze-running, or operant behaviour reinforced by a single schedule of reward) on which drug effects are tested. The choice of such a single criterion is likely to be arbitrary, and may well be insufficiently sensitive or reproducible to reveal drug effects. In human studies, a single performance or behavioural pattern may frequently be a composite of different components in different subjects. The participants in this conference, therefore, recommended very strongly that studies of drug effects on behaviour should include a spectrum of behavioural criteria, from which the effects on fundamental components of behaviour, rather than on specific responses, could be deduced. For example, a drug such as morphine probably acts on 'fundamental well-springs of behaviour' rather than on any particular type of behaviour (p. 145), so that a single test will usually fail to differentiate its action from that of other drugs. Moreover, effects of a drug on a given behaviour should be studied at all stages of its development: acquisition, maintenance and extinction. Behaviour is easiest to measure during the mainten-

ance phase because it is then most stable. For the same reason, it may also be most resistant to modification by drugs in this stage, and possibly the main effect of a given drug may be on acquisition or extinction of the behaviour.

Similarly, a single neurophysiological measure in a single species is of little value in explaining the behavioural effects of drugs, because a single measure usually does not permit conclusions about the basic mechanisms which are affected. For example, a reduction in amplitude of evoked potentials in the reticular formation may result from either the desynchronizing effect of a drug such as amphetamine, or an inhibition of neuronal responsiveness as by a barbiturate. The problem is compounded by individual and species differences so that an effect, for example, of chlordiazepoxide on interhippocampal responses in the cat may shed little light on its actions in man, who does not show such responses. In addition, many studies have ignored the effects of the drugs being studied on peripheral mechanisms regulating blood pressure, body temperature, respiration and other physiological processes. As a result, the central neurophysiological effects may be obscured by irrelevant secondary effects. It was therefore agreed that physiological studies of drug effects should also include as many different types of measure as possible, under conditions which maintain normal physiological homeostasis.

Biochemical test systems are usually more specifically defined, but even in this type of work there are important methodological problems. The tissue usually must be destroyed or disrupted in order to permit the isolation of an enzyme or other tissue constituent for biochemical study. An effect of a given drug on such a test system may then bear little relevance to the action of the drug in the living organism. It would therefore be desirable to study the effect of a given drug on a given biochemical process not in whole brain or cortex alone, but in as many different portions of the brain as possible. This might give better insight into the relationship between biochemical effects and functional effects of the drug.

(b) Choice of drug parameters. Again, at all levels of study, there was agreement that valid conclusions about the nature of a drug effect cannot be drawn from experiments in which a single drug dose or concentration is used. Full dose-effect curves should be obtained wherever possible so that biphasic or other complex patterns are not overlooked. This is particularly important in relation to large doses, which may provoke diffuse nonspecific toxic responses. An understanding of basic mechanisms of drug action can be obtained only at doses which do not produce such diffuse response.

Related to this question are certain response parameters which may be important in the lower dose range. The relation of the dose to the time course of onset and duration of action may be as informative as that to the maximum intensity of effect. In other cases, the drug effect may be principally upon the variability of a given response rather than upon its mean value.

(c) Terminology. Parallel with, and perhaps mutually reinforcing, the inadequacies of experimental methods noted above, are certain strongly entrenched inadequacies of terminology. A particularly good example is provided by the terms 'stimulant' and 'depressant.' It was pointed out eloquently and forcefully (p. 68) that these terms are meaningless without some indication of *what* is stimulated or depressed. For almost every drug one may point out some functions which are stimulated and others which are simultaneously depressed. Yet the terms, in common and scientific usage, have acquired a certain vague connotation which *appears* to be generally understood. As a result, they continue to be widely used, and were employed frequently during this conference, even after their inadequacy had been pointed out and agreed upon!

The concept of toxicity in general, and behavioural toxicity in particular, proved equally hard to define, despite the wide use of these terms. Indeed, no agreement was reached upon a satisfactory definition.

These examples pointed up the advisability of using, as much as possible, strictly descriptive operational terms, rather than terms which imply either value judgments, or mechanistic concepts which have not been proven (see pp.186-187).

Differences and Similarities among Addictive Drugs

It was generally agreed that, in a wide variety of behavioural and neurophysiological tests, opiate and phenothiazine tranquillizers give very similar results, despite the fact that they are poles apart in terms of their potential for producing dependence. Barbiturates, meprobamate, chlordiazepoxide, other hypnosedatives and volatile anesthetic agents constitute another group which differ from the former group but resemble each other fairly closely, especially at low doses. Alcohols generally resemble this latter group in their actions, but there is much less experimental evidence available on them. The only *single* test system so far encountered which differentiates between the effects of chlorpromazine and morphine in experimental animals is the test of analgesia to a heat stimulus applied to the tail or feet.

There are, however, differences among members of the same group, and there was no agreement reached, for example, on whether or not the long-acting and short-acting barbiturates differ from each other in any fundamental action. It was recognized that long-acting barbiturates do not produce euphoria and pose virtually no problem in relation to drug dependence, but there was disagreement as to whether or not this was entirely due to the time characteristics of their action.

To some extent, at least, the emphasis on similarities or on differences among the members of a group of drugs may depend on the personal interest of the investigator. Until basic mechanisms are better defined, the same results can be viewed in different ways.

Biochemical and Physiological Correlates of Drug Action

The consensus of opinion was that known neurophysiological and neurochemical processes are still very far from explaining all but a few of the simpler behavioural phenomena. Correspondingly, the known biochemical and physiological actions of drugs offer very little explanation of their effects on the living subject. This fact is shown in various ways.

For example, attempts to localize the primary sites of action of various drugs within the nervous system, in a way that might explain the behavioural effects, have so far been virtually unsuccessful. One of the difficulties has been that attempts at localization have been mainly in terms of anatomical regions, such as cerebral cortex or thalamus, rather than of functional systems or pathways. It is likely that drugs do not act on specific regions but on specific types of neuron or even on specific types of synapse. In the same region, cells which are side-by-side may show different drug responses because they have different functional characteristics.

Studies of drug distribution in the brain are of little help in localizing the sites of action. In a few instances, such as those phenothiazines which are effective against motion sickness, the drug distribution coincides with the physiological evidence on probable site of action. Specificity of action of these drugs may then depend upon their specific localization, even though the basic cellular action of the drugs might be fairly non-specific. In most other cases, however, it is impossible to see any correlation between distribution and effects. The localization of action may then depend upon the localization of particular susceptible cells or structures, even though the drug is diffusely distributed.

It was agreed that biochemical studies of drug effects so far are relevant only at the cellular level and cannot yet explain any of the known effects on integrated neurophysiological or behavioural functions. However, it seems highly probable that the effects of barbiturates, ethanol, phenothiazines, etc., on brain respiration, high energy phosphate compounds and intermediary metabolism are all secondary to the drug effects on the excitability of neuronal cell membranes. There is a pressing need for fundamental biochemical and biophysical research on the nature of the excitation process, before drug effects on it can be understood and perhaps differentiated.

Other drug effects which may have more relation to integrated nervous function are those exerted upon the formation, storage, release and degradation of possible neurotransmitter substances, including acetylcholine, noradrenaline, dopamine, serotonin and gamma-aminobutyrate. There was considerable discussion, but no agreement, about the role of acetylcholine as a central transmitter substance, and questions were raised about the similarity or dissimilarity between peripheral and central synaptic mechanisms. It was evident that much more knowledge is required about the roles of these transmitter substances in different parts of the brain before drug effects on the metabolism of these substances in isolated tissues can be interpreted in relation to brain function. The suggestion was made that the problem might be tackled in reverse. Certain functional systems and pathways can be identified *in vivo* by mapping the points at which specific electrical or chemical stimulation produces specific behaviour. If drug effects on these functional systems can be identified, then biochemical search can be narrowed to the changes produced in these systems.

Two traditional views about the actions of alcohol and barbiturates were discussed in some detail, and found to be unsupported by experimental evidence. The first is the dogma that alcohol acts first upon the cerebral cortex and affects subcortical systems only at higher concentrations. Detailed studies reported by Dr. Himwich showed that some cortical areas were more sensitive to ethanol, and others less sensitive, than the midbrain reticular formation. Again, it is necessary to think in terms of drug effects on functional systems rather than on 'levels' of brain organization. The second view to be questioned is that alcohol and barbiturates act to remove cortical inhibitory influences on behaviour. Evidence was presented to the effect that barbiturates did not specifically affect an imposed inhibition of operant response in pigeons, and ethanol did not abolish transcortical inhibitory influences in mammals. This may be another example of terminological problems, with 'inhibition' meaning different things in different types of investigation, but the traditional claim is, nevertheless, not proven experimentally.

Basic Mechanisms of Dependence and Addiction

There was full agreement concerning the almost complete lack of knowledge about basic neural and behavioural mechanisms involved in the development and maintenance of addictive patterns of behaviour. A comprehensive model was proposed (pp. 25-30) which can be tested experimentally at various levels from cellular chemistry to responses of integrated organisms. However, attention was drawn to many major gaps in knowledge and to areas in which caution must be exercised to avoid erroneous interpretations in the study of addiction.

One of these areas is the question of carry-over effects. The development of dependence or addiction obviously involves some cumulative change resulting from repeated administration of a drug, but the carry-over might be subthreshold with respect to a particular type of measurement, yet still be significant when added to the effect of a new dose. Again, the choice of neural parameters to be studied in relation to the development of dependence is still quite arbitrary. Most attention has been devoted to changes in the metabolism of neurotransmitter substances and of RNA and protein, but the rationale of selection is not always clear. There is also a tendency to forget that the sequence drug—cell change—behavioural change is not unidirectional and that behavioural changes selected for study may themselves modify cellular processes and so affect the drug response.

Other gaps in knowledge relate to the selection of criteria of drug effect. Highly specific behaviour patterns, such as imprinting, which bear some superficial resemblance to addictive behaviour in man, seem unlikely to provide any real insight into the question. Addiction, in man, is related to certain ill-defined euphorigenic properties of various drugs, but so far no animal test has been found which correlates with these properties. It was urged very strongly that a major research effort be directed to the problem of developing a simple predictive test of addictive liability which could be applied early in the development of new psychoactive drugs. The butyrophenones provide an illustration of how the same starting material (meperidine) can yield two derivatives, one morphine-like and one chlorpromazine-like, differing markedly in their properties according to the tests used in selecting them and 'shaping' their development. An effective test for addictive potential would be an invaluable addition to the selection process.

At the physiological and biochemical level there is virtually no knowledge of the nature of drug dependence. Is acutely acquired tolerance of the nervous system fundamentally different from chronic tolerance? What changes in neural pathways underlie the acquisition of tolerance? What cellular mechanisms are involved? Is the process exclusively within the central nervous system, or are peripheral tissue changes significantly involved in higher organisms? Except for a small scattering of studies, these questions are still almost unexplored, and there is an obvious need for much research into them. Some cellular changes related to drug dependence could conceivably be studied indirectly: for example, if it requires the synthesis of new receptors or new enzymes involved in the metabolism of transmitter substances, then puromycin or other inhibitors of protein synthesis should block the development of dependence. However, it was pointed out that such experiments may be subject to many serious errors of interpretation because puromycin, or similar agents, may have many other direct and indirect effects on the nervous system apart from the inhibition of protein synthesis.

Finally, it was recognized that individuals differ markedly in their susceptibility to drug dependence, and that research should be directed also to the reasons for such difference. Genetic selection studies in animals, aimed at separating strains with high and low tolerance to a given drug or group of drugs, might be of some value in clarifying the neural mechanisms and the predisposing factors related to acquisition of tolerance and dependence.

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